

NOVEL GPCR-LIKE PROTEINS AND NUCLEIC ACIDS ENCODING SAME

RELATED APPLICATIONS

5 This application claims priority from Provisional Applications U.S.S.N. 60/240704,
filed 16-Oct-2000; Provisional Applications U.S.S.N. 60/262159, filed 17-Jan-2001;
Provisional Applications U.S.S.N. 60/263340, filed 22-Jan-2001; Provisional Applications
U.S.S.N. 60/264118, filed 25-Jan-2001; Provisional Applications U.S.S.N. 60/308203, filed
27-Jul-2001; Provisional Applications U.S.S.N. 60/243497, filed 26-Oct-2000; Provisional
10 Applications U.S.S.N. 60/244542, filed 31-Oct-2000; Provisional Applications U.S.S.N.
60/269031, filed 15-Feb-2001; Provisional Applications U.S.S.N. 60/245484, filed 03-Nov-
2000; Provisional Applications U.S.S.N. 60/255017, filed 12-Dec-2000; Provisional
Applications U.S.S.N. 60/263216, filed 22-Jan-2001; and Provisional Applications U.S.S.N.
60/268225, filed 12-Feb-2001; each of which is incorporated by reference in its entirety.

15 FIELD OF THE INVENTION

The invention generally relates to novel GPCR1, GPCR2, GPCR3, GPCR4, GPCR5,
GPCR6, GPCR7, GPCR8, GPCR9, GPCR10, GPCR11 and GPCR12 nucleic acids and
polypeptides encoded therefrom. More specifically, the invention relates to nucleic acids
encoding novel polypeptides, as well as vectors, host cells, antibodies, and recombinant
20 methods for producing these nucleic acids and polypeptides.

BACKGROUND OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides. More particularly,
the invention relates to nucleic acids encoding novel G-protein coupled receptor (GPCR)
polypeptides, as well as vectors, host cells, antibodies, and recombinant methods for
25 producing these nucleic acids and polypeptides.

SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of nucleic acid sequences encoding novel polypeptides. The novel nucleic acids and polypeptides are referred to herein as GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, GPCR7, GPCR8, GPCR9, GPCR10, GPCR11 and GPCR12 nucleic acids and polypeptides. These nucleic acids and polypeptides, as well as derivatives, homologs, analogs and fragments thereof, will hereinafter be collectively designated as "GPCRX" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated GPCRX nucleic acid molecule encoding a GPCRX polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids disclosed in SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96. In some embodiments, the GPCRX nucleic acid molecule will hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of a GPCRX nucleic acid sequence. The invention also includes an isolated nucleic acid that encodes a GPCRX polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide comprising the amino acid sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a GPCRX nucleic acid (*e.g.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96) or a complement of said oligonucleotide.

Also included in the invention are substantially purified GPCRX polypeptides (SEQ ID NOS: 2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 or 97). In certain embodiments, the GPCRX polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human GPCRX polypeptide.

The invention also features antibodies that immunoselectively bind to GPCRX polypeptides, or fragments, homologs, analogs or derivatives thereof.

In another aspect, the invention includes pharmaceutical compositions that include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a GPCR_X nucleic acid, a GPCR_X polypeptide, or an antibody specific for a GPCR_X polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a GPCR_X nucleic acid, under conditions allowing for expression of the GPCR_X polypeptide encoded by the DNA. If desired, the GPCR_X polypeptide can then be recovered.

In another aspect, the invention includes a method of detecting the presence of a GPCR_X polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby identifying the GPCR_X polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of a GPCR_X.

Also included in the invention is a method of detecting the presence of a GPCR_X nucleic acid molecule in a sample by contacting the sample with a GPCR_X nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to a GPCR_X nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of a GPCR_X polypeptide by contacting a cell sample that includes the GPCR_X polypeptide with a compound that binds to the GPCR_X polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, , developmental diseases; MHCII and III diseases (immune diseases); taste and scent detectability disorders; Burkitt's lymphoma; corticoneurogenic disease; signal transduction pathway disorders; metabolic pathway disorders; retinal diseases including those involving

photoreception; cell growth rate disorders; cell shape disorders; metabolic disorders; feeding disorders; control of feeding; the metabolic syndrome X; wasting disorders associated with chronic diseases; obesity; potential obesity due to over-eating or metabolic disturbances; potential disorders due to starvation (lack of appetite); diabetes; noninsulin-dependent diabetes mellitus (NIDDM1); infectious disease; bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2); pain; cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer); cancer-associated cachexia; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; Crohn's disease; multiple sclerosis; Albright Hereditary Osteodystrophy; angina pectoris; myocardial infarction; ulcers; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders; including anxiety; schizophrenia; manic depression; delirium; dementia; neurodegenerative disorders; Alzheimer's disease; severe mental retardation; Dentatorubro-pallidoluysian atrophy (DRPLA); Hypophosphatemic rickets; autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome; immune disorders; Adrenoleukodystrophy; Congenital Adrenal Hyperplasia; Hemophilia; Hypercoagulation; Idiopathic thrombocytopenic purpura; autoimmune disease; immunodeficiencies; transplantation; Von Hippel-Lindau (VHL) syndrome; Stroke; Tuberous sclerosis; hypercalcaemia; Cerebral palsy; Epilepsy; Lesch-Nyhan syndrome; Ataxia-telangiectasia; Leukodystrophies; Behavioral disorders; Addiction; Neuroprotection; Cirrhosis; Transplantation; Systemic lupus erythematosus; Emphysema; Scleroderma; ARDS; Renal artery stenosis; Interstitial nephritis; Glomerulonephritis; Polycystic kidney disease; Systemic lupus erythematosus; Renal tubular acidosis; IgA nephropathy; Cardiomyopathy; Atherosclerosis; Congenital heart defects; Aortic stenosis; Atrial septal defect (ASD); Atrioventricular (A-V) canal defect; Ductus arteriosus; Pulmonary stenosis; Subaortic stenosis; Ventricular septal defect (VSD); valve diseases; Scleroderma; fertility; Pancreatitis; Endocrine dysfunctions; Growth and reproductive disorders; Inflammatory bowel disease; Diverticular disease; Leukodystrophies; Graft versus host; Hyperthyroidism; Endometriosis; hematopoietic disorders and/or other pathologies and disorders of the like. The therapeutic can be, *e.g.*, a GPCR nucleic acid, a GPCR polypeptide, or a GPCR-specific antibody, or biologically-active derivatives or fragments thereof.

For example, the compositions of the present invention will have efficacy for treatment of patients suffering from the diseases and disorders listed above and/or other pathologies and disorders.

5 The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding GPCR_X may be useful in gene therapy, and GPCR_X may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering the diseases and disorders listed above and/or other pathologies and disorders.

10 The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, diseases and disorders listed above and/or other pathologies and disorders and those disorders related to cell signal processing and metabolic pathway modulation. The method includes contacting a test compound with a GPCR_X polypeptide and
15 determining if the test compound binds to said GPCR_X polypeptide. Binding of the test compound to the GPCR_X polypeptide indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including the diseases
20 and disorders listed above and/or other pathologies and disorders or other disorders related to cell signal processing and metabolic pathway modulation by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a GPCR_X nucleic acid. Expression or activity of GPCR_X polypeptide is then measured in the test animal, as is expression or activity
25 of the protein in a control animal which recombinantly-expresses GPCR_X polypeptide and is not at increased risk for the disorder or syndrome. Next, the expression of GPCR_X polypeptide in both the test animal and the control animal is compared. A change in the activity of GPCR_X polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

30 In yet another aspect, the invention includes a method for determining the presence of or predisposition to a disease associated with altered levels of a GPCR_X polypeptide, a GPCR_X nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes

measuring the amount of the GPCR_X polypeptide in a test sample from the subject and comparing the amount of the polypeptide in the test sample to the amount of the GPCR_X polypeptide present in a control sample. An alteration in the level of the GPCR_X polypeptide in the test sample as compared to the control sample indicates the presence of or predisposition to a disease in the subject. Preferably, the predisposition includes diseases and disorders listed above and/or other pathologies and disorders. Also, the expression levels of the new polypeptides of the invention can be used in a method to screen for various cancers as well as to determine the stage of cancers.

In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject a GPCR_X polypeptide, a GPCR_X nucleic acid, or a GPCR_X-specific antibody to a subject (e.g., a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes the diseases and disorders listed above and/or other pathologies and disorders.

In yet another aspect, the invention can be used in a method to identify the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode novel polypeptides. The novel nucleic acids and their encoded polypeptides are

referred to individually as GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, GPCR7, GPCR8, GPCR9, GPCR10, GPCR11 and GPCR12. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "GPCRX".

The novel GPCRX nucleic acids of the invention include the GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, GPCR7, GPCR8, GPCR9, GPCR10, GPCR11 and GPCR12 nucleic acids, or a fragment, derivative, analog or homolog thereof. The novel GPCRX proteins of the invention include the GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, GPCR7, GPCR8, GPCR9, GPCR10, GPCR11 and GPCR12 proteins, or a derivative, analog or homolog thereof. The individual GPCRX nucleic acids and proteins are described below. Within the scope of this invention is a method of using these nucleic acids and peptides in the treatment or prevention of a disorder related to cell signaling or metabolic pathway modulation.

The GPCRX proteins of the invention have a high homology to the 7tm_1 domain (PFam Acc. No. pfam00001). The 7tm_1 domain is from the 7 transmembrane receptor family, which includes a number of different proteins, including, for example, serotonin receptors, dopamine receptors, histamine receptors, adrenergic receptors, cannabinoid receptors, angiotensin II receptors, chemokine receptors, opioid receptors, G-protein coupled receptor (GPCR) proteins, olfactory receptors (OR), and the like. Some proteins and the Protein Data Base Ids/gene indexes include, for example: 5-hydroxytryptamine receptors (See, e.g., PMIM 112821, 8488960, 112805, 231454, 1168221, 398971, 112806); rhodopsin (129209); G protein-coupled receptors (119130, 543823, 1730143, 132206, 137159, 6136153, 416926, 1169881, 136882, 134079); gustatory receptors (544463, 462208); c-x-c chemokine receptors (416718, 128999, 416802, 548703, 1352335); opsins (129193, 129197, 129203); and olfactory receptor-like proteins (129091, 1171893, 400672, 548417).

Because of the close homology among the members of the GPCRX family, proteins that are homologous to any one member of the family are also largely homologous to the other members, except where the sequences are different as shown below.

The similarity information for the GPCRX proteins and nucleic acids disclosed herein suggest that GPCR1-GPCR12 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic

acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon.

G-Protein Coupled Receptor proteins ("GPCRs") have been identified as a large family of G protein-coupled receptors in a number of species. These receptors share a seven transmembrane domain structure with many neurotransmitter and hormone receptors, and are likely to underlie the recognition and G-protein-mediated transduction of various signals. Human GPCR generally do not contain introns and belong to four different gene subfamilies, displaying great sequence variability. These genes are dominantly expressed in olfactory epithelium. See, e.g., Ben-Arie et al., *Hum. Mol. Genet.* 1994 3:229-235; and, Online Mendelian Inheritance in Man ("OMIM") entry # 164342 (<http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?>).

The olfactory receptor ("OR") gene family constitutes one of the largest GPCR multigene families and is distributed among many chromosomal sites in the human genome. See Rouquier et al., *Hum. Mol. Genet.* 7(9):1337-45 (1998); Malnic et al., *Cell* 96:713-23 (1999). Olfactory receptors constitute the largest family among G protein-coupled receptors, with up to 1000 members expected. See Vanderhaeghen et al., *Genomics* 39(3):239-46 (1997); Xie et al., *Mamm. Genome* 11(12):1070-78 (2000); Issel-Tarver et al., *Proc. Natl. Acad. Sci. USA* 93(20):10897-902 (1996). The recognition of odorants by olfactory receptors is the first stage in odor discrimination. See Krautwurst et al., *Cell* 95(7):917-26 (1998); Buck et al., *Cell* 65(1):175-87 (1991). Many ORs share some characteristic sequence motifs and have a central variable region corresponding to a putative ligand binding site. See Issel-Tarver et al., *Proc. Natl. Acad. Sci. USA* 93:10897-902 (1996).

Other examples of seven membrane spanning proteins that are related to GPCRs are chemoreceptors. See Thomas et al., *Gene* 178(1-2):1-5 (1996). Chemoreceptors have been identified in taste, olfactory, and male reproductive tissues. See *id.*; Walensky et al., *J. Biol. Chem.* 273(16):9378-87 (1998); Parmentier et al., *Nature* 355(6359):453-55 (1992); Asai et al., *Biochem. Biophys. Res. Commun.* 221(2):240-47 (1996).

The GPCR_X nucleic acids of the invention encoding GPCR-like proteins include the nucleic acids whose sequences are provided herein, or fragments thereof. The invention also includes mutant or variant nucleic acids any of whose bases may be changed from the corresponding base shown herein while still encoding a protein that maintains its GPCR-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

The GPCR_X proteins of the invention include the GPCR-like proteins whose sequences are provided herein. The invention also includes mutant or variant proteins any of whose residues may be changed from the corresponding residue shown herein while still encoding a protein that maintains its GPCR-like activities and physiological functions, or a functional fragment thereof. The invention further encompasses antibodies and antibody fragments, such as F_{ab} or (F_{ab})₂, that bind immunospecifically to any of the proteins of the invention.

The GPCR_X nucleic acids and proteins are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the GPCR (or olfactory-receptor) like protein may be useful in gene therapy, and the receptor-like protein may be useful when administered to a subject in need thereof. The nucleic acids and proteins of the invention are also useful in potential therapeutic applications used in the treatment of developmental diseases; MHCII and III diseases (immune diseases); taste and scent detectability disorders; Burkitt's lymphoma; corticoneurogenic disease; signal transduction pathway disorders; metabolic pathway disorders; retinal diseases including those involving photoreception; cell growth rate disorders; cell shape disorders; metabolic disorders; feeding disorders; control of feeding; the metabolic syndrome X; wasting disorders associated with chronic diseases; obesity; potential obesity due to over-eating or metabolic disturbances;

potential disorders due to starvation (lack of appetite); diabetes; noninsulin-dependent diabetes mellitus (NIDDM1); infectious disease; bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2); pain; cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer); cancer-associated cachexia; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; Crohn's disease; multiple sclerosis; Albright Hereditary Osteodystrophy; angina pectoris; myocardial infarction; ulcers; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders; including anxiety; schizophrenia; manic depression; delirium; dementia; neurodegenerative disorders;

5 Alzheimer's disease; severe mental retardation; Dentatorubro-pallidoluysian atrophy (DRPLA); Hypophosphatemic rickets; autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome; immune disorders; Adrenoleukodystrophy; Congenital Adrenal Hyperplasia; Hemophilia; Hypercoagulation; Idiopathic thrombocytopenic purpura; autoimmune disease;

10 immunodeficiencies; transplantation; Von Hippel-Lindau (VHL) syndrome; Stroke; Tuberous sclerosis; hypercalcaemia; Cerebral palsy; Epilepsy; Lesch-Nyhan syndrome; Ataxia-telangiectasia; Leukodystrophies; Behavioral disorders; Addiction; Neuroprotection; Cirrhosis; Transplantation; Systemic lupus erythematosus; Emphysema; Scleroderma; ARDS; Renal artery stenosis; Interstitial nephritis; Glomerulonephritis; Polycystic kidney disease;

15 Systemic lupus erythematosus; Renal tubular acidosis; IgA nephropathy; Cardiomyopathy; Atherosclerosis; Congenital heart defects; Aortic stenosis ; Atrial septal defect (ASD); Atrioventricular (A-V) canal defect; Ductus arteriosus; Pulmonary stenosis ; Subaortic stenosis; Ventricular septal defect (VSD); valve diseases; Scleroderma; fertility; Pancreatitis; Endocrine dysfunctions; Growth and reproductive disorders; Inflammatory bowel disease;

20 Diverticular disease; Leukodystrophies; Graft versus host; Hyperthyroidism; Endometriosis; hematopoietic disorders and/or other pathologies and disorders. Other GPCR-related diseases and disorders are contemplated.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and

30 antagonist compounds. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the anti-GPCR_X antibody compositions of the

present invention will have efficacy for treatment of patients suffering from the diseases and disorders listed above, as well as other related or associated pathologies. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

GPCR1

A GPCR-like protein of the invention, referred to herein as GPCR1, is an Olfactory Receptor (“OR”)-like protein. Some members of the Olfactory Receptor-Like Protein Family end up localized at the cell surface, where they exhibit activity. Therefore it is likely that these novel GPCR1 proteins are available at the appropriate sub-cellular localization and hence accessible for the therapeutic uses described in this application.

Six alternative novel GPCR1 nucleic acids and encoded polypeptides are provided, namely GPCR1a, GPCR1b, GPCR1c, GPCR1d, GPCR1e and GPCR1f. The GPCR1 proteins are predicted to be a likely Type IIIb membrane protein.

GPCR1a

In one embodiment, the disclosed GPCR1 variant is the novel GPCR1a (alternatively referred to as GMAC073079_A), which includes the 964 nucleotide sequence (SEQ ID NO:1) shown in Table 1A. The disclosed GPCR1a open reading frame (“ORF”) begins at an ATG initiation codon at nucleotides 19-21 and terminates at a TGA codon at nucleotides 958-960. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 1A, and the start and stop codons are in bold letters.

Table 1A. GPCR1 nucleotide sequence (SEQ ID NO:1).

GGCCCCATACTGTGGATCATGGCAAATCTGAGCCAGCCCTCCGAATTTGTCCTCTTGGGCTTCTCCTCC TTTGGTGAGCTGCAGGCCCTTCTGTATGGCCCCCTTCCTCATGCTTTATCTTCTCGCCTTCATGGGAAAC ACCATCATCATAGTTATGGTCATAGCTGACACCCACCTACATACACCCATGTACTTCTTCTGGGCAAT TTTTCCCTGCTGGAGATCTTGTAACCATGACTGCAGTGCCAGGATGCTCTCAGACCTGTTGGTCCCC CACAAAGTCATTACCTTCACTGGCTGCATGGTCCAGTTCTACTTCCACTTTTCCCTGGGGTCCACCTCC TTCCTCATCTGACAGACATGGCCCTTGATCGCTTGTGGCCATCTGCCACCCACTGCGCTATGGCACT CTGATGAGCCGGGTATGTGTGCCAGCTGGCTGGGGCTGCCTGGGCAGCTCCTTTCTAGCCATGGTA CCCACTGTCTCTCCCGAGCTCATCTTGATTACTGCCATGGCGACGTCATCAACCACTTCTTGTGAC AATGAACCTCTCCTGCAGTTGTCATGCTCTGACACTCGCCTGTTGGAATTCTGGGACTTTCTGATGGCC TTGACCTTTGTCTCAGCTCCTTCTGGTGACCCTCATCTCCTATGGCTACATAGTGACCACTGTGCTG
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CGGATCCCCTCTGCCAGCAGCTGCCAGAAGGCTTTCTCCACTTGC GGGTCTCACCTCACACTGGTCTTC
 ATCGGCTACAGTAGTACCATCTTTCTGTATGTCAGGCCTGGCAAAGCTCACTCTGTGCAAGTCAGGAAG
 GTCGTGGCCTTGGTGACTTCAGTTCTACCCCTTTCTCAATCCCTTTATCCTTACCTTCTGCAATCAG
 ACAGTTAAACAGTGCTACAGGGGCAGATGCAGAGGCTGAAAGGCCTTTGCAAGGCACAATGATGAG

In the present invention, the GPCR1a target sequence was subjected to the exon linking process to confirm the sequence. These procedures provide the sequences reported below, which are designated GPCR1b (also referred to as AC073079_da1), GPCR2 (also referred to as AC073079_da2), and GPCR1c (also referred to as AC073079_da3).

The sequence of GPCR1a was derived by laboratory cloning of cDNA fragments, by *in silico* prediction of the sequence. The cDNA fragments covering either the full length of the DNA sequence, or part of the sequence, or both, were cloned. *In silico* prediction was based on sequences available in CuraGen's proprietary sequence databases or in the public human sequence databases, and provided either the full length DNA sequence, or some portion thereof.

The disclosed GPCR1 of this invention maps to chromosome 1. Chromosome localization information was assigned using OMIM, the electronic northern bioinformatic tool implemented by CuraGen Corporation, public ESTs, public literature references and/or genomic clone homologies. This was executed to derive the chromosomal mapping of the SeqCalling assemblies, Genomic clones, literature references and/or EST sequences that were included in the invention.

The disclosed GPCR1a polypeptide (SEQ ID NO:2) encoded by SEQ ID NO:1 has 313 amino acid residues, has a molecular weight of 34900.65 Daltons, and is presented in Table 1B using the one-letter amino acid code. The Signal P, Psort and/or Hydropathy results predict that GPCR1a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, the GPCR1a protein is localized to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the microbody (peroxisome) with a certainty of 0.3000. The most likely cleavage site for a GPCR1a peptide is between amino acids 47 and 48, *i.e.*, at the dash in the sequence VIA-DT.

Table 1B. Encoded GPCR1 protein sequence (SEQ ID NO:2).

MANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLAFMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEI

LVTMTAVPRMLSDDLPHKVITFTGCMVQFYFHFSLGSTSFLIILTMALDRFVAICHPLRYGTLMSRAM
CVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQLSCSDTRLLEFWDFLMALTFVLS
SFLVTLISYGYIVTTVLRIPSASSCQKAFSTCGSHLTLVFIGYSSITFLYVRPGKAHSVQVRKVVALVT
SVLTPFLNPFILTFQNTVKTVLQGGMQRLKGLCKAQ

GPCR1b

In one embodiment, the disclosed GPCR1 variant is the novel GPCR1b (alternatively referred to as AC073079_da1), which includes the 971 nucleotide sequence (SEQ ID NO:3) shown in Table 1C. The disclosed GPCR1b open reading frame (“ORF”) begins at an ATG initiation codon at nucleotides 30-32 and terminates at a TGA codon at nucleotides 963-965. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 1C, and the start and stop codons are in bold letters.

Table 1C. GPCR1b nucleotide sequence (SEQ ID NO:3).

CCCCATACTGTGGATCATGGCAAGGCACAATGATGAGCCAGCCCTCCGAATTTGTCCTCTTGGGCTTCTCCT
CCTTTGGTGAGCTGCAGGCCCTTCTGTATGGCCCCCTTCCTCATGCTTTATCTTCTCGCCTTCATGGGAAACA
CCATCATCATAGTTATGGTTCATAGCTGACACCCACCTACATACACCCATGTACTTCTTCTGGGCAATTTT
CCCTGCTGGAGATCTTGGTAACCATGACTGCAGTGCCCAGGATGCTCTCAGACCTGTTGGTCCCCCACAAAG
TCATTACCTTCACTGGCTGCATGGTCCAGTTCTACTTCCACTTTTCCCTGGGGTCCACCTCCTTCCTCATCC
TGACAGACATGGCCCTTGATCGCTTTGTGGCCATCTGCCACCCACTGCGCTATGGCACTCTGATGAGCCGGG
CTATGTGTGTCCAGCTGGCTGGGGCTGCCTGGGCAGCTCCTTCTAGCCATGGTACCCACTGTCTCTCCC
GAGCTCATCTTGATTACTGCCATGGCGACGTCAACCACTTCTTCTGTGACAATGAACCTCTCCTGCAGT
TGTCATGCTCTGACACTCGCCTGTTGGAATTCTGGGACTTTCTGATGGCCATGACCTTTGTCCTCAGCTCCT
TCCTGGTGACCTCATCTCATATGGCTACATAGTGACCACTGTGCTGCGGATCCCTCTGCCAGCAGCTGCC
AGAAGGCTTTCTCCACTTGCGGGTCTCACCTCACACTGGTCTTCATCGGCTACAGTAGTACCATCTTTCTGT
ATGTCAGGCCTGGCAAAGCTCACTCTGTGCAAGTCAGGAAGGTCGTGGCCTTGGTGACTTCAGTTCTCACCC
CCTTTCTCAATCCCTTTATCCTTACCTTCTGCAATCAGACAGTTAAACAGTGCTACAGGGGCAGATGCAGA
GGCTGAAAGGCCTTTGCAAGGCACAATGATGAGCC

The disclosed GPCR1b polypeptide (SEQ ID NO:4) encoded by SEQ ID NO:3 has 311 amino acid residues, a molecular weight of 34751.56 Daltons, and is presented in Table 1D using the one-letter amino acid code. The Signal P, Psort and/or Hydropathy results predict that GPCR1b has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, the GPCR1b protein is localized to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the microbody (peroxisome) with a certainty of 0.3000. The most likely cleavage site for a GPCR1b peptide is between amino acids 47 and 48, *i.e.*, at the dash in the sequence VIA-DT.

Table 1D. Encoded GPCR1b protein sequence (SEQ ID NO:4).

MMSQPSEFVLLGFSSFGELQALLYGPFLMLYLLAFMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILV
TMTAVPRMLSDDLVPKHVITFTGCMVQFYFHFSLGSTSFLILTDMALDRFVAICHPLRYGTLMSRAMCV
QLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQLSCSDTRLLEFWDFLMAMTFVLSSF
LVTLLISYGYIVTTVLRIPSASSCQKAFSTCGSHLTLVFIGYSSTIFLYVRPGKAHSVQVRKVVALVTSV
LTPFLNPFILTFQNTQVKTVLQGMQRLKGLCKAQ

In a search of sequence databases, it was found, for example, that the GPCR1b nucleic acid sequence of this invention has 543 of 882 bases (61%) identical to a gb:GENBANK- ID:AF102523| acc:AF102523.1 mRNA from Mus musculus (Mus musculus olfactory receptor C6 gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 137 of 301 amino acid residues (45%) identical to, and 199 of 301 amino acid residues (66%) similar to, the 313 amino acid residue ptrn:SPTREMBL-ACC:Q9Z1V0 protein from Mus musculus (Mouse) (OLFACTORY RECEPTOR C6).

GPCR1c

In one embodiment, the disclosed GPCR1 variant is the novel GPCR1c (alternatively referred to as AC073079_da3), which includes the 971 nucleotide sequence (SEQ ID NO:5) shown in Table 1E. The disclosed GPCR1c open reading frame (“ORF”) begins at an ATG initiation codon at nucleotides 30-32 and terminates at a TGA codon at nucleotides 963-965. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 1E, and the start and stop codons are in bold letters.

Table 1E. GPCR1c nucleotide sequence (SEQ ID NO:5).

CCCCATACTGTGGATCATGGCAAGGCACAATGATGAGCCAGCCCTCCGAATTTGTCCTCTTGGGCTTCTCCT
CCTTTGGTGAGCTGCAGGCCCTTCTGTATGGCCCTTCCTCATGCTTTATCTTCTCGCCTTCATGGGAAACA
CCATCATCATAGTTATGGTCATAGCTGACACCCACCTACATACACCCATGTACTTCTTCCTGGGCAATTTTT
CCCTGCTGGAGATCTTGGTAACCATGACTGCAGTGCCAGGATGCTCTCAGACCTGTTGGTCCCCACAAAG
TCATTACCTTCACTGGCTGCATGGTCCAGTTCTACTTCCACTTTTCCCTGGGGTCCACCTCCTTCCTCATCC
TGACAGACATGGCCCTTGATCGCTTTGTGGCCATCTGCCACCCACTGCGCTATGGCACTCTGATGAGCCGGG
CTATGTGTGCCAGCTGGCTGGGCTGCCTGGGCAGCTCCTTTTCCTAGCCATGGTACCCACTGTCTCTCCC
GAGCTCATCTTGATTACTGCCATGGCGACGTCATCAACCACTTCTTCTGTGACAATGAACCTCTCCTGCAGT
TGTCATGCTCTGACACTCGCCTGTTGGAATTCTGGGACTTTCTGATGGCCTTGACCTTTGTCCTCAGCTCCT
TCCTGGTGACCCTCATCTCCTATGGCTACATAGTGACCACTGTGCTGCGGATCCCCTCTGCCAGCAGCTGCC
AGAAGGCTTCTCCACTTGCGGGTCTCACCTCACACTGGTCTTCATCGGCTACAGTAGTACCATCTTTCTGT
ATGTCAGGCTGGCAAAGCTCACTCTGTGCAAGTCAGGAAGGTCGTGGCCTTGGTGACTTCAGTTCTCACCC
CCTTTCTCAATCCCTTTATCCTTACCTTCTGCAATCAGACAGTTAAACAGTGCTACAGGGGCAGATGCAGA
GGCTGAAAGGCCTTTGCAAGGCACAATGATGAGCC

The disclosed GPCR1c polypeptide (SEQ ID NO:6) encoded by SEQ ID NO:5 has 311 amino acid residues, a molecular weight of 34733.52 Daltons, and is presented in Table 1F using the one-letter amino acid code. The Signal P, Psort and/or Hydropathy results predict that GPCR1c has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, the GPCR1c protein is localized to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the microbody (peroxisome) with a certainty of 0.3000. The most likely cleavage site for a GPCR1c peptide is between amino acids 47 and 48, *i.e.*, at the dash in the sequence VIA-DT.

Table 1F. Encoded GPCR1c protein sequence (SEQ ID NO:6).

MMSQPSSEFVLLGFSSFGELQALLYGPFLMLYLLAFMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILV TMTAVPRMLSDLLVPHKVITFTGCMVQFYFHFSLGSTSFLILTDMALDRFVAICHPLRYGTLMsRAMCV QLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQLSCSDTRLLEFWDFLMALTFVLSSSF LVTLLISYGYIVTTVLRIPSASSCQKAFSTCGSHLTLVFIGYSSTIFLYVRPGKAHSVQVRKVVALVTSV LTPFLNPFILTFCNQTVKTVLQGQMQRLLKGLCKAQ

In a search of sequence databases, it was found, for example, that the GPCR1c nucleic acid sequence of this invention has 589 of 932 bases (63%) identical to a gb:GENBANK-ID:AF101760| acc:AF101760.1 mRNA from Gorilla gorilla (Gorilla gorilla isolate PPOR1E2 olfactory receptor gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 137 of 301 amino acid residues (45%) identical to, and 199 of 301 amino acid residues (66%) similar to, the 313 amino acid residue ptnr:SPTREMBL-ACC:Q9Z1V0 protein from Mus musculus (Mouse) (OLFACTORY RECEPTOR C6).

GPCR1d

In one embodiment, the disclosed GPCR1 variant is the novel GPCR1d (alternatively referred to as BA113A10_B_da1), which includes the 992 nucleotide sequence (SEQ ID NO:7) shown in Table 1G. The disclosed GPCR1d open reading frame ("ORF") begins at an GTC codon at nucleotides 3-5 and terminates at a TGA codon at nucleotides 987-989. Putative untranslated region downstream from the termination codon is underlined in Table 1G, and the initial and stop codons are in bold letters.

Table 1G. GPCR1 nucleotide sequence (SEQ ID NO:7).

CTGTCTTTTGTCTCTTGCATGCAGGGCCCCATACTGTGGATCATGGCAAATCTGAGCCAGCCCTCCGAAT
 TTGTCCTCTTGGGCTTCTCCTCCTTTGGTGAGCTGCAGGCCCTTCTGTATGGCCCCCTCCTCATGCTTTATC
 TTCTCGCCTTCATGGGAAACACCATCATCATAGTTATGGTCATAGCTGACACCCACCTACATACACCCATGT
 ACTTCTTCTGGGCAATTTTCCCTGCTGGAGATCTTGGTAACCATGACTGCAGTGCCAGGATGCTCTCAG
 ACCTGTTGGTCCCCACAAAGTCATTACCTTCACTGGCTGCATGGTCCAGTTCTACTTCCACTTTTCCCTGG
 GGTCACCTCCTTCTCATCCTGACAGACATGGCCCTTGATCGCTTGTGGCCATCTGCCACCCACTGCGCT
 ATGGCACTCTGATGAGCCGGGCTATGTGTGTCCAGCTGGCTGGGGCTGCCTGGGCAGCTCCTTTCCTAGCCA
 TGGTACCCACTGTCCTCTCCCAGCTCATCTTGATTACTGCCATGGCGACGTCAACCACTTCTTCTGTG
 ACAATGAACCTCTCCTGCAGTTGTCTGCTGACACTCGCCTGTTGGAATTCTGGGACTTCTGATGGCCT
 TGACCTTTGTCCTCAGCTCCTTCTGGTGACCTCATCTCCTATGGCTACATAGTGACCACTGTGCTGCGGA
 TCCCCCTGCGCAGCAGCTGCCAGAAGGCTTTCTCCACTTGCGGGTCTACCTCACACTGGTCTTCATCGGCT
 ACAGTAGTACCATCTTTCTGTATGTGAGGCCTGGCAAAGCTCACTCTGTGCAAGTCAGGAAGTTCGTGGCCT
 TGGTGACTTCAGTTCTACCCCTTTCTCAATCCCTTTATCCTTACCTTCTGCAATCAGACAGTTAAACAG
 TGCTACAGGGGCAGATGCAGAGGCTGAAAGGCCCTTGCAAGGCACAATGATGAGCC

In certain embodiments, the open reading frame of the disclosed GPCR1d nucleic acid is an incomplete cDNA fragment, and it is contemplated that the ORF extends upstream (*i.e.*, in the 5' direction) of the sequence provided in SEQ ID NO:7. It is further contemplated that a complete ORF would include an in-frame ATG codon as the start codon.

The disclosed GPCR1d polypeptide (SEQ ID NO:8) encoded by SEQ ID NO:7 has 327 amino acid residues, a molecular weight of 36526.60 Daltons, and is presented in Table 1H using the one-letter amino acid code. The Signal P, Psort and/or Hydropathy results predict that GPCR1d has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. In alternative embodiments, the GPCR1d protein is localized to the Golgi body with a certainty of 0.4600, the endoplasmic reticulum (membrane) with a certainty of 0.3700, or the microbody (peroxisome) with a certainty of 0.1000. The most likely cleavage site for a GPCR1d peptide is between amino acids 63 and 64, *i.e.*, at the dash in the sequence VIA-DT.

Table 1H. Encoded GPCR1d protein sequence (SEQ ID NO:8).

VFCFSCMQGPILWIMANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLAFMGNTIIIVMVIADTHLHT
 PMYFFLGNFSLLEILVTMTAVPRMLSDLLVPHKVITFTGCMVQFYFHFSLGSTSFLILTDMALDRFVAI
 CHPLRYGTILMSRAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQLSCSDTRLL
 EFWDFLMALTFVLSSFLVTLISYGYIVTTVLRIPSASSCQKAFSTCGSHLTLVFIGYSSTIFLYVRPGK
 AHSVQVRKVVALVTSVLTPFLNPFILTFCNQTVKTVLQGQMQRLLKGLCKAQ

In a search of sequence databases, it was found, for example, that the GPCR1d nucleic acid sequence of this invention has 555 of 904 bases (61%) identical to a gb:GENBANK-

ID:AF102523 |acc:AF102523.1 mRNA from Mus musculus (Mus musculus olfactory receptor C6 gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 140 of 308 amino acid residues (45%) identical to, and 203 of 308 amino acid residues (65%) similar to, the 313 amino acid residue ptmr:SPTREMBL-ACC:Q9Z1V0 protein from Mus musculus (Mouse) (OLFACTORY RECEPTOR C6).

GPCR1e

In one embodiment, the disclosed GPCR1 variant is the novel GPCR1e (alternatively referred to as BA113A10_B_da3), which includes the 971 nucleotide sequence (SEQ ID NO:9) shown in Table 1I. The disclosed GPCR1e open reading frame ("ORF") begins at an ATG initiation codon at nucleotides 24-26 and terminates at a TGA codon at nucleotides 936-938. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 1I, and the start and stop codons are in bold letters.

Table 1I. GPCR1e nucleotide sequence (SEQ ID NO:9).

TGCAAGGCCCCATACTGTGGAT**AT**GGCAAATCTGAGCCAGCCCTCCGAATTGTCCTCTTGGGCTTCTCCTCCTTTGGTG
AGCTGCAGGCCCTTCTGTATGGCCCCCTCCTCATGCTTTATCTTCTCGCCTTCATGGGAAACACCATCATAGTTATGG
TCATAGCTGACACCCACCTACATACACCCATGTACTTCTTCTGGGCAATTTTCCCTGCTGGAGATCTTGGTAACCATGA
CTGCAGTGCCCAGGATGCTCTCAGACCTGTTGGTCCCCCAAAAGTCATTACCTTCACTGGCTGCATGGTCCAGTTCTACT
TCCACTTTTCCCTGGGGTCCACCTCCTTCCCTCATCTGACAGACATGGCCCTTGATCGCTTTGTGGCCATCTGCCACCCAC
TGCGCTATGGCACTCTGATGAGCCGGGCTATGTGTGCCAGCTGGCTGGGGCTGCCTGGGCAGCTCCTTTCTAGCCATGG
TACCACTGTCTCTCCGAGCTCATCTTGATTACTGCCATGGCGACGTCATTAACCACTTCTTCTGTGACAATGAACCTC
TCCTGCAGTTGTCTGACACTCGCCTGTTGGAATCTGGGACTTTCTGATGGTCTTGACCTTTGTCTCAGCTCCT
TCCTGGTGACCTCATCTCTATGGCTACATAGTGACCACTGTGCTGCGGATCCCCTGCGCAGCAGCTGCCAGAAGGCTT
TCTCCACTTGCGGGTCTCACCTCACCTGGTCTTCATCGGTACAGTAGTACCATCTTCTGTATGTGAGGCCTGGCAAAG
CTCACTCTGTGCAAGTCAGGAAGGTCGTGGCCTTGGTGACTTCAGTTCTACCCCTTCTCAATCCCTTTATCCTTACCT
TCTGCAATCAGACAGTTAAACAGTGCTACAGGGGCAGATGCAGAGGCTGAAAGGCCTTTGCAAGGCACAAT**GATGAGCC**

The disclosed GPCR1e polypeptide (SEQ ID NO:10) encoded by SEQ ID NO:9 has 313 amino acid residues, a molecular weight of 34928.71 Daltons, and is presented in Table 1J using the one-letter amino acid code. The Signal P, Psort and/or Hydropathy results predict that GPCR1e has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, the GPCR1e protein is localized to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the microbody (peroxisome) with a certainty of 0.3000. The most likely cleavage

site for a GPCR1e peptide is between amino acids 49 and 50, *i.e.*, at the dash in the sequence VIA-DT.

Table 1J. Encoded GPCR1e protein sequence (SEQ ID NO:10).

MANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLAFMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILV TMTAVPRMLSDDLVPKHVITFTGCMVQFYFHFSLGSTSLILTDALDRFVAICHPLRYGTLMSRAMCVQL AGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFCDNEPLLQLSCSDTRLLEFWDFLMVLTFVLSSFLVTL ISYGYIVTTVLRI PSASSCQKAFSTCGSHLTIVFIGYSSTIFLYVRPGKAHSVQVRKVVALVTSVLTPFLN PFILTFCNQTVKTVLQGQMQRLLKGLCKAQ
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In a search of sequence databases, it was found, for example, that the GPCR1e nucleic acid sequence of this invention has 555 of 904 bases (61%) identical to a gb:GENBANK-ID:AF102523|acc:AF102523.1 mRNA from *Mus musculus* (*Mus musculus* olfactory receptor C6 gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 139 of 308 amino acid residues (45%) identical to, and 202 of 308 amino acid residues (65%) similar to, the 313 amino acid residue ptnr:SPTREMBL-ACC:Q9Z1V0 protein from *Mus musculus* (Mouse) (OLFACTORY RECEPTOR C6).

GPCR1f

In one embodiment, the disclosed GPCR1 variant is the novel GPCR1f (alternatively referred to as CG50303_02), which includes the 992 nucleotide sequence (SEQ ID NO:11) shown in Table 1K. The disclosed GPCR1 open reading frame (“ORF”) begins at an ATG initiation codon at nucleotides 21-23 and terminates at a TAG codon at nucleotides 954-956. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 1K, and the start and stop codons are in bold letters.

Table 1K. GPCR1 nucleotide sequence (SEQ ID NO:11).

CTGTCTTTTGTCTTCTTGCATGCAAGGCCCCATACTGTGGATCATGGCAAATCTGAGCCAGCCCTCCG AATTTGTCCTCTTGGGCTTCTCCTCCTTTGGTGAGCTGCAGGCCCTTCTGTATGGCCCCCTTCTCATGC TTTATCTTCTCGCCTTCATGGGAAACACCATCATCATAGTTATGGTCATAGCTGACACCCACCTACATA CACCCATGTACTTCTTCTGGGCAATTTTCCCTGCTGGAGATCTTGGTAACCATGACTGCAGTGCCCA GGATGCTCTCAGACCTGTTGGTCCCCACAAAGTCATTACCTTCACTGGCTGCATGGTCCAGTTCTACT TCCACTTTTCCCTGGGGTCCACCTCCTTCCCTCATCTGACAGACATGGCCCTTGATCGCTTTGTGGCCA TCTGCCACCCACTGCGCTATGGCACTCTGATGAGCCGGGCTATGTGTGTCCAGCTGGCTGGGGCTGCCT GGGCAGCTCCTTTCTAGCCATGGTACCCACTGTCTCTCCCGAGCTCATCTTGATTACTGCCATGGCG ACGTCATTAAACCACTTCTTCTGTGACAATGAACCTCTCCTGCAGTTGTATGCTCTGACACTCGCCTGT TGGAATTCTGGGACTTTCTGATGGTCTTGACCTTTGTCTCAGCTCCTTCTGGTGACCTCATCTCCT ATGGCTACATAGTGACCACTGTGCTGCGGATCCCTCTGCCAGCAGCTGCCAGAAGGCTTTCTCACTT GCGGGTCTCACCTCACACTGGTCTTCATCGGCTACAGTAGTACCATCTTCTGTATGTGAGGCTGGCA AAGCTCACTCTGTGCAAGTCAGGAAGGTCGTGGCCTTGGTGACTTCAGTTCTACCCCTTTCTCAATC
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CCTTTATCCTTACCTTCTGCAATCAGACAGTTAAACAGTGCTACAGGGGCAGATGTAGAGGCTGAAAG
GCCTTTGCAAGGCACAATGATGAGCC

The disclosed GPCR1f polypeptide (SEQ ID NO:12) encoded by SEQ ID NO:11 has 311 amino acid residues, a molecular weight of 34741.50 Daltons, and is presented in Table 1L using the one-letter amino acid code. The Signal P, Psort and/or Hydropathy results predict that GPCR1f has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. In alternative embodiments, the GPCR1f protein is localized to the Golgi body with a certainty of 0.4600, the endoplasmic reticulum (membrane) with a certainty of 0.3700, or the microbody (peroxisome) with a certainty of 0.1000. The most likely cleavage site for a GPCR1f peptide is between amino acids 57 and 58, *i.e.*, at the dash in the sequence VIA-DT.

Table 1L. Encoded GPCR1f protein sequence (SEQ ID NO:12).

MQGPILWIMANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLAFMGNTIIIVMVIADTHLHTPMYFFL
GNFSLLEILVTMTAVPRMLSDLLVPHKVITFTGCMVQFYFHFSLGSTSFLILTDMALDRFVAICHPLRY
GTLMSRAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQLSCSDTRLLEFWDFL
MVLTFVLSSFLVTLISYGYIVTTVLRI PSASSCQKAFSTCGSHLTLVFIGYSSITFLYVRPGKAHSVQV
RKVVALVTSVLTPFLNPFILTFCNQTVKTVLQGQM

In a search of sequence databases, it was found, for example, that the GPCR1f nucleic acid sequence of this invention has 560 of 912 bases (61%) identical to a gb:GENBANK- ID:AF102523|acc:AF102523.1 mRNA from *Mus musculus* (*Mus musculus* olfactory receptor C6 gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 139 of 301 amino acid residues (46%) identical to, and 199 of 301 amino acid residues (66%) similar to, the 313 amino acid residue ptmr:SPTREMBL-ACC:Q9Z1V0 protein from *Mus musculus* (Mouse) (OLFACTORY RECEPTOR C6).

GPCR1 Clones

Unless specifically addressed as GPCR1a, GPCR1b, GPCR1c, GPCR1d, GPCR1e or GPCR1f, any reference to GPCR1 is assumed to encompass all variants. Residue differences between any GPCRX variant sequences herein are written to show the residue in the "a" variant, the residue position with respect to the "a" variant, and the residue in any one of the "b" through "f" variants.

Additional SNP variants of GPCR1 are disclosed in Example 3. Sequence differences between the GPCR1 clones are shown in the ClustalW alignment in Table 1L, with variant positions marked with a "o" above the variant sequence.

Public and proprietary sequence databases were searched for protein sequences with
5 homology to GPCR1 using BLASTP software. In all BLAST alignments herein, the "E-value"
or "Expect" value is a numeric indication of the probability that the aligned sequences could
have achieved their similarity to the BLAST query sequence by chance alone, within the
database that was searched. For example, the probability that the subject sequence ("Sbjct"),
e.g., patp acc no. AAG71691 *Homo sapiens* olfactory receptor polypeptide, retrieved from the
10 GPCR1 BLAST analysis of the proprietary PatP database matched the Query GPCR1
sequence purely by chance is 7.2×10^{-166} , as shown in Table 1M. The Expect value (E) is a
parameter that describes the number of hits one can "expect" to see just by chance when
searching a database of a particular size. It decreases exponentially with the Score (S) that is
assigned to a match between two sequences of a database of comparable complexity.
15 Essentially, the E value describes the random background noise that exists for matches
between sequences.

The E value is used as a convenient way to create a significance threshold for reporting
results. The default value used for blasting is typically set to 0.0001. In BLAST 2.0, the E
value is also used instead of the P value (probability) to report the significance of matches.
20 For example, an E value of one assigned to a hit can be interpreted as meaning that in a
database of the current size one might expect to see one match with a similar score simply by
chance. An E value of zero means that one would not expect to see any matches with a similar
score simply by chance. See, e.g., <http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/>.
Occasionally, a string of X's or N's will result from a BLAST search. This is a result of
25 automatic filtering of the query for low-complexity sequence that is performed to prevent
artifactual hits. The filter substitutes any low-complexity sequence that it finds with the letter
"N" in nucleotide sequence (e.g., "NNNNNNNNNN") or the letter "X" in protein sequences
(e.g., "XXX"). Low-complexity regions can result in high scores that reflect compositional
bias rather than significant position-by-position alignment (Wootton and Federhen, *Methods*
30 *Enzymol* 266:554-571, 1996).

The disclosed GPCR1 amino acid sequence has 210 of 314 amino acid residues (66%) identical to, and 250 of 314 residues (79%) positive with, the *Mus musculus* 315 amino acid residue olfactory receptor protein (ptnr: SPTREMBL-ACC:Q9QZ17)($E = 3.1e^{-106}$).

The amino acid sequence of GPCR1 had high homology to other proteins as shown in Table 1M.

Table 1M. BLASTX results from PatP database for GPCR1			
		High Score	Smallest Sum Prob P (N)
Sequences producing High-scoring Segment Pairs:			
patp:AAG71691	Human olfactory receptor polypeptide	1615	7.2e-166
patp:AAG71685	Human olfactory receptor polypeptide	776	5.8e-77
patp:AAG72507	Human OR-like polypeptide query sequence	776	5.8e-77
patp:AAG71951	Human olfactory receptor polypeptide	743	1.8e-73
patp:AAG72408	Human OR-like polypeptide query sequence	743	1.8e-73

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of this invention has 555 of 904 bases (61%) identical to a gb:GENBANK-ID:AF102523|acc:AF102523.1 mRNA from *Mus musculus* (*Mus musculus* olfactory receptor C6 gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 140 of 308 amino acid residues (45%) identical to, and 203 of 308 amino acid residues (65%) similar to, the 313 amino acid residue ptnr:SPTREMBL-ACC:Q9Z1V0 protein from *Mus musculus* (Mouse) (Olfactory Receptor C6).

GPCR1 also has homology to the proteins shown in the BLASTP data in Table 1F.

Table 1N. GPCR1 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
TREMBLNEW-ACC:AAK69553	OLFACTORY RECEPTOR SDOLF - Homo sapiens	280	144/269 (53%)	195/269 (72%)	8.5e-77
TREMBLNEW-ACC:AAK70859	ODORANT RECEPTOR ORZ6 - Mus musculus (Mouse)	314	139/307 (45%)	203/307 (66%)	9.1e-73
SPTREMBL-ACC:Q9Z1V0	OLFACTORY RECEPTOR C6 - Mus musculus (Mouse)	313	140/308 (45%)	203/308 (65%)	6.6e-70
TREMBLNEW-ACC:CAC43444	(NOVEL 7 TRANSMEMBRANE RECEPTOR (RHODOPSIN FAMILY) (OLFACTORY RECEPTOR LIKE) PROTEIN SIMILAR TO HUMAN HS6M1-21) - Mus musculus (Mouse)	317	137/298 (45%)	196/298 (65%)	1.6e-66
SPTREMBL-ACC:Q9EPG2	M51 OLFACTORY RECEPTOR - Mus musculus (Mouse)	314	132/298 (44%)	188/298 (63%)	5.5e-66

A multiple sequence alignment is given in Table 10, with the GPCR1a-GPCR1f proteins being shown on lines 1 through 6, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 1N. This BLASTP data is displayed graphically in the ClustalW in Table 10.

Table 10. ClustalW Analysis of GPCR1

1. SEQ ID NO:2, GPCR1a
2. SEQ ID NO:4, GPCR1b
3. SEQ ID NO:6, GPCR1c
4. SEQ ID NO:8, GPCR1d
5. SEQ ID NO:10, GPCR1e
6. SEQ ID NO:12, GPCR1f
7. SEQ ID NO:13, AAK70859 ODORANT RECEPTOR ORZ6
8. SEQ ID NO:14, Q9Z1V0 OLFACTORY RECEPTOR C6
9. SEQ ID NO:15, CAC43444
10. SEQ ID NO:16, Q9EPG2 M51 OLFACTORY RECEPTOR
11. SEQ ID NO:17, AAK69553 OLFACTORY RECEPTOR SDOLF

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GPCR1a	-----MANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLA	36
GPCR1b	-----MMSQPSEFVLLGFSSFGELQALLYGPFLMLYLLA	34
GPCR1c	-----MMSQPSEFVLLGFSSFGELQALLYGPFLMLYLLA	34
GPCR1d	VFCFSCMQGPILWIMANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLA	50
GPCR1e	-----MANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLA	36
GPCR1f	-----MQGPILWIMANLSQPSEFVLLGFSSFGELQALLYGPFLMLYLLA	44
AAK70859	-----MMDNLSAIEFCLLGPFGSQELHYLLFAIFFFFYSVT	37
Q9Z1V0	-----MANSTTVIEFLLGLSDACELQVLLFLGLLTYFLI	36
CAC43444	-----MEGKNOTAPSEFLLGFDHLNELQYLLFTIFFLTYCT	38
Q9EPG2	-----MLDMNITLVSEFLLVGFPAPWLQILLFFIFLVVYMLI	38
AAK69553	-----M	1
GPCR1a	FMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILVTMTAVPRMLSDDLVP	86
GPCR1b	FMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILVTMTAVPRMLSDDLVP	84
GPCR1c	FMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILVTMTAVPRMLSDDLVP	84
GPCR1d	FMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILVTMTAVPRMLSDDLVP	100
GPCR1e	FMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILVTMTAVPRMLSDDLVP	86
GPCR1f	FMGNTIIIVMVIADTHLHTPMYFFLGNFSLLEILVTMTAVPRMLSDDLVP	94
AAK70859	LLGNMVIITVVCVDKRLQSPMYFFLGNLSLLEILVTITIVPLMLWGLLP	87
Q9Z1V0	LLGNFLIITFLLVDRRLYTPMYFFLRNFAMLEIWFTSVIFPKMLTNTIT	85
CAC43444	LGGNVFIIVTIADSHLHTPMYFFLGNLALLDICYTTNVPOMMVHLLSE	88
Q9EPG2	IAENLVIIIFTWSTGSLHKPMYFFLSSMSFLEIWIYVSVIVPKMLDGFLQ	88
AAK69553	LLGNLAIITSFCLDSRLHSPMYFFLGNFSLLEIVVTSTIVVHRMLADLLST	51
GPCR1a	-HKVITFTGCMVQFYFHFSLGSTSFLLITDMALDRFVAICHPLRYGTLMS	135
GPCR1b	-HKVITFTGCMVQFYFHFSLGSTSFLLITDMALDRFVAICHPLRYGTLMS	133
GPCR1c	-HKVITFTGCMVQFYFHFSLGSTSFLLITDMALDRFVAICHPLRYGTLMS	133
GPCR1d	-HKVITFTGCMVQFYFHFSLGSTSFLLITDMALDRFVAICHPLRYGTLMS	149
GPCR1e	-HKVITFTGCMVQFYFHFSLGSTSFLLITDMALDRFVAICHPLRYGTLMS	135
GPCR1f	-HKVITFTGCMVQFYFHFSLGSTSFLLITDMALDRFVAICHPLRYGTLMS	143
AAK70859	GKQTIISLNGCIAQLFLYLALGTEFAVLGAMAYDRVVAICNPLRYSVIMN	137
Q9Z1V0	GKTIISLLGCFLOALYFFLGLTEFFLLAVMSFDRVVAICNPLRYATIMS	135
CAC43444	-KKIISYGGCVTQLFAFIFFVGSECLLAAMAYDRVAICKPLRYSFIMN	137
Q9EPG2	-RRHISFTGCMTOLYFFISLACTECVLAAMAYDRVAICHPLRYPVIMN	137
AAK69553	-HKTHSLAKCTQSEFFSLGSAFLILMVMAFDRVAICHPLRYPTITN	100

GPCR1a	RAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQL	185
GPCR1b	RAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQL	183
GPCR1c	RAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQL	183
GPCR1d	RAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQL	199
GPCR1e	RAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQL	185
GPCR1f	RAMCVQLAGAAWAAPFLAMVPTVLSRAHLDYCHGDVINHFFCDNEPLLQL	193
AAK70859	SRTCWVVMVSWMFGFLSELPVYATFQFTCKSNLHSHFCDRGQLLKL	187
Q9Z1V0	KRYCVQLVFCWMSGLLLIVPSSIVFQQPTCGPNLINHFFCDNEPLLQL	185
CAC43444	KALCSWLAASCWTCGFLNSVLHTVLTFLHPGNNQINFFCDIPPLLIL	187
Q9EPG2	TVYCMOLMALSYFSGFVSVVKYFISHVAFCSNVMNHFFCDISPILLKL	187
AAK69553	GPVCVLIVVACWVVGFLSIVSPTLQKTRLWFCGPNLIHGYFCDSAPLLKL	150

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GPCR1a	SCSDTRLLEFWDFLMALTFVLSSFLVTLISYGYIVTTVLRIPSASSCQKA	235
GPCR1b	SCSDTRLLEFWDFLMALTFVLSSFLVTLISYGYIVTTVLRIPSASSCQKA	233
GPCR1c	SCSDTRLLEFWDFLMALTFVLSSFLVTLISYGYIVTTVLRIPSASSCQKA	233
GPCR1d	SCSDTRLLEFWDFLMALTFVLSSFLVTLISYGYIVTTVLRIPSASSCQKA	249
GPCR1e	SCSDTRLLEFWDFLMVLTFLVSSFLVTLISYGYIVTTVLRIPSASSCQKA	235
GPCR1f	SCSDTRLLEFWDFLMVLTFLVSSFLVTLISYGYIVTTVLRIPSASSCQKA	243
AAK70859	SCNETFLTERILFIMAFILVGSLLPTLVSYTYILSTILKIPSASGRKA	237
Q9Z1V0	ICADTSLVEELGFEVIANFSLGLAVTATCYGHITLILHIPSASAKERKA	235
CAC43444	SCGDTSLNELALLSIGILIGWLPFLCIILSYLYILSTILRIRSEGRQKA	237
Q9EPG2	ACKDMSTAEVLVDFALAHVILVFPILITVLSYVYIVSTILRIPSTQGRKA	237
AAK69553	ACSDTRHIERMDLFLSLILFVLITMLLITLSYILIVA AVLHIPSSSCQKA	200

GPCR1a	FSTCGSHLTLVFIGYSSTIFLYVRPGK-AHSVQVRKVVALVTSVLTTPFLN	284
GPCR1b	FSTCGSHLTLVFIGYSSTIFLYVRPGK-AHSVQVRKVVALVTSVLTTPFLN	282
GPCR1c	FSTCGSHLTLVFIGYSSTIFLYVRPGK-AHSVQVRKVVALVTSVLTTPFLN	282
GPCR1d	FSTCGSHLTLVFIGYSSTIFLYVRPGK-AHSVQVRKVVALVTSVLTTPFLN	298
GPCR1e	FSTCGSHLTLVFIGYSSTIFLYVRPGK-AHSVQVRKVVALVTSVLTTPFLN	284
GPCR1f	FSTCGSHLTLVFIGYSSTIFLYVRPGK-AHSVQVRKVVALVTSVLTTPFLN	292
AAK70859	FSTCASHFTFVWVIGYGICFLYVRP-KQTQAAEYNRVASLLVSVVTPFLN	286
Q9Z1V0	FSTCSSHITVVSIFYGSCIFLYVRSGKNGQGEDHNKVVALNIVVTPFLN	285
CAC43444	FSTCASHLLVILYYGSAIFTYVRP-ISSYSLEKDRITSVLYSVFTPMLN	286
Q9EPG2	FSTCASHLTVVIIYYTAMIFMYVRP-RAIASFNSNKLISAVYAVLTPMLN	286
AAK69553	FSTCASHLTVVVLGYGSAIFLYVRPGK-GHSTYLNKAVAMVTAMVTPFLN	249

oooooooo

GPCR1a	PFILTFCNQTVKTVLQGMORLKGLCKAQ--	313
GPCR1b	PFILTFCNQTVKTVLQGMORLKGLCKAQ--	311
GPCR1c	PFILTFCNQTVKTVLQGMORLKGLCKAQ--	311
GPCR1d	PFILTFCNQTVKTVLQGMORLKGLCKAQ--	327
GPCR1e	PFILTFCNQTVKTVLQGMORLKGLCKAQ--	313
GPCR1f	PFILTFCNQTVKTVLQGM-----	311
AAK70859	PFIFTLRNDKVKEALRDGVRCCLLLRD---	314
Q9Z1V0	PFIYTLRNKQVKQVFRHVSFKFKFSQT---	313
CAC43444	PFIYALRNKDIKEAVKAIGRWOPPVFSSDM	317
Q9EPG2	PFIYCLRNREVKDAIKKTLGGGCFLLC---	314
AAK69553	PFIFTFRNEKVKEVIEDVTKRIFLGDPAAACR	280

DOMAIN

The results indicate that the GPCR1 protein contains the following protein domain (as defined by Interpro): domain name 7tm_1 7 transmembrane receptor (rhodopsin family).

- DOMAIN results for GPCR1 were collected from the Conserved Domain Database (CDD)

with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections.

As discussed below, all GPCR_X proteins of the invention contain significant homology to the 7_{tm}_1 domain. This indicates that the GPCR_X sequence has properties similar to those of other proteins known to contain this 7_{tm}_1 domain and similar to the properties of these domains. The 254 amino acid domain termed 7_{tm}_1 (SEQ ID NO:18)(Pfam acc. no. 00001), a seven transmembrane receptor (rhodopsin family), is shown in Table 1P

Table 1P. 7_{tm}_1, 7 transmembrane receptor domain (SEQ ID NO:18)

GNLLVILVILRTKKLRTPNTIFLLNLAVADLLFLLTLPWALYYLVGGDWVFGDALCKLVGALFVNGYASILLTASIDRYL
AIVHPLRYRRIRTPRAKVLILLVWVLALLSLPPLFSWLRVVEEGNTTVCLIDFPEESVKRSYVLLSTLVGFVLPPLLVLVC
YTRILRTLKRARSQRLKRRSSSERKAAKMLLVVVVFVLCWLPYHIVLLLSLCLLSIWRVLPALLITLWLAYVNSCLNPI
IY

The encoded GPCR₁ polypeptide was identified as a member of the G protein receptor family due to the presence of a signature consensus sequence (SEQ ID NO:19) shown in Table 1Q below.

Table 1Q. G-protein coupled receptors signature domain (SEQ ID NO:19)

Entry Name	G PROTEIN RECEPTOR
Entry Type	PATTERN
Primary Accession Number	PS00237
Created / Last Updated	01-APR-1990 / 01-JUL-1998
Description	G-protein coupled receptors signature.
Pattern	[GSTALIVMFYWC]-[GSTANCPDE]-{EDPKRH}-x(2)- [LIVMNQGA]-x(2)-[LIVMFT]-[GSTANC]- [LIVMFYWSTAC]-[DENH]-R-[FYWCSH]-x(2)-[LIVM].

Table 1R lists the domain description from DOMAIN analysis results against GPCR₁. This indicates that the GPCR₁ sequence has properties similar to those of other proteins known to contain this domain as well as to the 254 amino acid 7_{tm} domain (SEQ ID NO:39). For Table 1R and all successive DOMAIN sequence alignments, fully conserved single residues are indicated by black shading and “strong” semi-conserved residues are indicated by grey shading. The “strong” group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

The DOMAIN results are listed in Table 1R with the statistics and domain description. An alignment of GPCR1 residues 41-290 (SEQ ID NO:2) with the full 7tm_1 domain, residues 1-254 (SEQ ID NO:18), are shown in Table 1R.

Table 1R. Domain Analysis of GPCR1				
PSSMs producing significant alignments:			Score (bits)	E value
7tm_1 (InterPro) 7 transmembrane receptor (rhodopsin family)			94.6	4.3e-29
7tm_1	1	*->GNlLVilvilrtkklrtptnifilNLAvADLLflltlppwalyylv	47	
		GN ++i+ ++ +l+tp+++f++N ++ +L++ t +p +l+ l+		
GPCR1	53	GNTIIIVMVIADTHLHTPMYFFLGNFSLLEILVTMTAVPRMLSDLLV	99	
7tm_1	48	gsedWpfGsalCklvtaldvvnmyaSillLtaISiDRYlAivhPlryrrr	97	
		++++ +C ++ ++ + + +S l Lt +++DR++AI+hPlry ++		
GPCR1	100	--PHKVITFTGCMVQFYFHFSLGSTSFLILTDMALDRFVAICHPLRYGTL	147	
7tm_1	98	rtsprrrAkvvillvWvlalllslPpllfswvktveegngtlnvntvCli	147	
		++ + ++ + +++W++ +l+ +P ++s ++ + +++ +n+++C+		
GPCR1	148	MS-RAMCVQLAGAAWAAPFLAMVPT-VLSRAHLDYCHGDV--INHFFCDN	193	
7tm_1	148	dfpeestasvstwlrsyvlstlvGflPlilvilvcYtrIlrtlr.....	192	
		+ ++s+ l+++ +l l + l +lv l+ Y+ I+ t+ + ++		
GPCR1	194	EPLLQLSCSDTRLLEFWDFLMALTFVLSSFLVTLISYGYIVTTVLripsa	243	
7tm_1	193	...kaaktllvvvvvFvLCWlPyfivllldtlc.lsiimsstCelervlp	239	
		++ + a+ ++ +++ v+ + i+l++++ + s ++		
GPCR1	244	sscQKAFSTCGSHLTLVFIGYSSTIFLYVRPGKaHS-----VQ	281	
7tm_1	240	tallvtlwLayvNscINPiY<-*	261	
		+ v+l+ +++ + lNP+I		
GPCR1	282	VRKVVALVTSVLTPFLNPFIL	302	

5

The rhodopsin-like GPCRs themselves represent a widespread protein family that includes hormone, neurotransmitter and light receptors, all of which transduce extracellular signals through interaction with guanine nucleotide-binding (G) proteins. Although their activating ligands vary widely in structure and character, the amino acid sequences of the receptors are very similar and are believed to adopt a common structural framework comprising 7 transmembrane (TM) helices. G-protein-coupled receptors (GPCRs) constitute a vast protein family that encompasses a wide range of functions (including various autocrine, paracrine and endocrine processes). They show considerable diversity at the sequence level, on the basis of which they can be separated into distinct groups. The term clan is used to describe the GPCRs, as they embrace a group of families for which there are indications of evolutionary relationship, but between which there is no statistically significant similarity in

sequence. The currently known clan members include the rhodopsin-like GPCRs, the secretin-like GPCRs, the cAMP receptors, the fungal mating pheromone receptors, and the metabotropic glutamate receptor family.

5 The homologies shown in the tables above indicates that the GPCR1 sequences of the invention have properties similar to those of other proteins known to contain this/these domain(s) as well as properties similar to the properties of these domains.

10 The Olfactory Receptor-like GPCR1 disclosed in this invention is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, 15 small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR1 is provided in Example 2. 20

25 The nucleic acids and proteins of GPCR1 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described above and further herein. The novel GPCR1 nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

30 These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. The disclosed GPCR1 protein has multiple hydrophilic regions, each of which can be used as an immunogen.

GPCR1a, GPCR1b and GPCR1e have similar hydropathy plots, and hence are predicted to have similar epitope locations. In one embodiment, a contemplated GPCR1 epitope for these variants is from about amino acids 1 to 20. In additional embodiments, these GPCR1 variants have epitopes that are from about amino acids 155 to 195, from about amino acids 230 to 240, from about amino acids 255 to 275 and from about amino acids 290 to the C-terminus.

In another embodiment, a contemplated GPCR1c epitope is from about amino acids 1 to 25, from about amino acids 50 to 65, from about amino acids 120 to 130, from about amino acids 155 to 190, from about amino acids 220 to 240, from about amino acids 250 to 275 and from about amino acids 280 to the C-terminus. In a further embodiment, a contemplated GPCR1d epitope is from about amino acids 1 to 20, from about amino acids 170 to 210, from about amino acids 245 to 255, from about amino acids 270 to 285 and from about amino acids 305 to the C-terminus. In a final embodiment, a contemplated GPCR1f epitope is from about amino acids 1 to 20, from about amino acids 165 to 210, from about amino acids 235 to 250, from about amino acids 260 to 280 and from about amino acids 300 to the C-terminus.

GPCR2

The disclosed novel GPCR2 (alternatively referred to herein as AC073079_da2) includes the 990 nucleotide sequence (SEQ ID NO:20) shown in Table 2A. A GPCR2 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 7-9 and ends with a TAG codon at nucleotides 937-939. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 2A, and the start and stop codons are in bold letters.

Table 2A. GPCR2 Nucleotide Sequence (SEQ ID NO:20)

GACTAAATGATGGACAACCACTCTAGTGCCACTGAATTCACCTTCTAGGCTTCCCTGGGTCCCAAGG
 ACTACACCACATTCTTTTGCTATATTCTTTTCTTCTATTTAGTGACATTAATGGGAAACACGGTCA
 TCATTGTGATTGTCTGTGTGGATAAACGCTCGCAGTCCCCCATGTATTTCTTCTCAGCCACCTCTCT
 ACCCTGGAGATCCTGGTCACAACCATAATTGTCCCATGATGCTTTGGGGATTGCTCTTCTGGGATG
 CAGACAGTATCTTTCTCTACATGTATCGCTCAACTTTTCTGTGGGACCATGGAGTTTGCATTACTTG
 GAGTGATGGCTGTGGACCGTTATGTGGCTGTGTGTAACCTTTGAGGTACAACATCATTATGAACAGC
 AGTACCTGTATTTGGGTGGTAATAGTGTCTATGGGTGTTTGGATTCTTTCTGAAATCTGGCCCATCTA
 TGCCACATTTCAAGTTTACCTTCCGCAAATCAAATTCATTAGACCATTTTACTGTGACCGAGGGCAAT
 TGCTCAAACCTGTCTGCGATAACACTCTTCTCACAGAGTTTATCCTTTTCTTAATGGCTGTTTTATT
 CTCATTGGTTCTTTGATCCCTACGATTGTCTCCTACACCTACATTATCTCCACCATCCTCAAGATCCC
 GTCAGCCTCTGGCCGGAGGAAAGCCTTCTCCACTTTTGCCTCCCACTTCACCTGTGTTGTGATTGGCT
 ATGGCAGCTGCTTGTCTCTACGTGAAACCAAGCAAACACAGGGAGTTGAGTACAATAAGATAGTT
 TCCTTGTGGTTTCTGTGTTAACCCCTTCTGAATCCTTTCATCTTACTCTTCGGATGACAAAGT

CAAAGAGGCCCTCCGAGATGGGATGAAACGCTGCTGTCAACTCCTGAAAGATTAGCTGTTCTGTAAGT
CAGTTT TAGGTGGTCCAAGCCTCAGGGTTAATTATTAA

A GPCR-like protein of the invention, referred to herein as GPCR2, is an Olfactory Receptor ("OR")-like protein. Some members of the Olfactory Receptor-Like Protein Family end up localized at the cell surface, where they exhibit activity. Therefore it is likely that these novel GPCR2 proteins are available at the appropriate sub-cellular localization and hence accessible for the therapeutic uses described in this application.

The GPCR2 polypeptide (SEQ ID NO:21) encoded by SEQ ID NO:20 is 310 amino acids in length, has a molecular weight of 35240.98 Daltons, and is presented using the one-letter amino acid code in Table 2B. The Signal P, Psort and/or Hydropathy results predict that GPCR2 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, the GPCR2 protein is localized to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the microbody (peroxisome) with a certainty of 0.3000. The most likely cleavage site for a GPCR1a peptide is between amino acids 40 and 41, *i.e.*, at the dash in the sequence LMG-NT. GPCR2 is predicted to be a likely Type IIb membrane protein. Additional SNP variants of GPCR2 are disclosed in Example 3.

Table 2B. GPCR2 protein sequence (SEQ ID NO:21)

MMDNHSSATEFHLLGFPQSQGLHHILFAIFFFFYLVTLMGNTVIIVIVCVDKRLQSPMYFFLSHLST
LEILVTTIIVPMMWLGLFLGCRQYLSLHVSLNFSCTMEFALLGVMAVDYVAVCNPLRYNIIMNS
STCIWVVIVSWVFGFLSEIWIYATFQFTFRKSNSLDHFYCDRGQLLKLSCDNTLLTEFILFLMAVF
ILIGSLIPTIVSYTYIISTILKIPSASGRKAFSTFASHFTCVVIGYGSCFLFLYVKPKQTQGVYENK
IVSLLVSVLTPLPESFHYSSDDKVKEALRDGMKRCCQLLKD

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of this invention has 302 of 343 bases (88%) identical to a gb:GENBANK-ID:HSHTPRX06| acc:X64982.1 mRNA from Homo sapiens (H.sapiens mRNA HTPCRX06 for olfactory receptor). The full amino acid sequence of the protein of the invention was found to have 134 of 306 amino acid residues (43%) identical to, and 189 of 306 amino acid residues (61%) similar to, the 313 amino acid residue ptrn:SPTREMBL-ACC:Q9Z1V0 protein from Mus musculus (Mouse) (OLFACTORY RECEPTOR C6).

The amino acid sequence of GPCR2 had high homology to other proteins as shown in Table 2E.

Table 2E. BLASTX results from PatP database for GPCR2

Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Prob P (N)
patp:AAG71689 Human olfactory receptor polypeptide	1554	2.1e-159
patp:AAG71971 Human olfactory receptor polypeptide	1207	1.2e-122
patp:AAG71969 Human olfactory receptor polypeptide	1128	2.9e-114
patp:AAG72438 Human OR-like polypeptide query sequence	1128	2.9e-114
patp:AAG71813 Human olfactory receptor polypeptide	996	2.8e-100

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of this invention has 543 of 882 bases (61%) identical to a gb:GENBANK-ID:AF102523|acc:AF102523.1 mRNA from Mus musculus (Mus musculus olfactory receptor C6 gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 137 of 301 amino acid residues (45%) identical to, and 199 of 301 amino acid residues (66%) similar to, the 313 amino acid residue ptnr:SPTREMBL-ACC:Q9Z1V0 protein from Mus musculus (Mouse) (OLFACTORY RECEPTOR C6).

GPCR2 also has homology to the proteins shown in the BLASTP data in Table 2F.

Table 2F. GPCR2 BLASTP results

Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
TREMBLNEW-ACC:AAK70859	ODORANT RECEPTOR ORZ6 Mus musculus (Mouse)	314	243/314 (77%)	272/314 (86%)	1.3e-126
SPTREMBL-ACC:Q9Z1V0	OLFACTORY RECEPTOR C6 Mus musculus (Mouse)	313	134/306 (43%)	189/306 (61%)	7.9e-58
SPTREMBL-ACC:Q9EPV0	M50 OLFACTORY RECEPTOR Mus musculus (Mouse)	316	129/300 (43%)	186/300 (62%)	2.1e-57
SPTREMBL-ACC:Q9EPG1	M50 OLFACTORY RECEPTOR Mus musculus (Mouse)	316	129/300 (43%)	186/300 (62%)	2.1e-57
SPTREMBL-ACC:Q9EPG2	M51 OLFACTORY RECEPTOR Mus musculus (Mouse)	314	125/302 (41%)	183/302 (60%)	2.8e-55

A multiple sequence alignment is given in Table 2G, with the GPCR2 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 2F. This BLASTP data is displayed graphically in the ClustalW in Table 2G.

Table 2G. ClustalW Analysis of GPCR2

- SEQ ID NO: 21, GPCR2
- SEQ ID NO: 22, TREMBLNEW-ACC:AAK70859 ODORANT RECEPTOR ORZ6
- SEQ ID NO: 23, SPTREMBL-ACC:Q9Z1V0 OLFACTORY RECEPTOR C6
- SEQ ID NO: 24, SPTREMBL-ACC:Q9EPV0 M50 OLFACTORY RECEPTOR
- SEQ ID NO: 25, SPTREMBL-ACC:Q9EPG1 M50 OLFACTORY RECEPTOR

6. SEQ ID NO: 26, SPTREMBL-ACC:Q9EPG2 M51 OLFACTORY RECEPTOR

GPCR2	--MMDNHSSATEFHLLGFPQSQGLDHHILFAIFFFFYLVTLMGNTVIIIVVC	49
AAK70859	--MMDNLSSATEFCLLGFPQSQGLDHYILFAIFFFFYSVTLLGNMVIILIVC	49
Q9Z1V0	--MANSTVTEFILLGLSDACBLQVLLIFLGFLTYFILLGNFIIFITL	48
Q9EPV0	--MENITNISEFILMGFPTAPWLQILLFSIFFFTYVFVLLNLVIIITVW	48
Q9EPG1	--MENITNISEFILMGFPTAPWLQILLFSIFFFTYVFVLLNLVIIITVW	48
Q9EPG2	MMDMNTLVSEFILVGFPTAPWLQILLFFIFLVVYMLIAENLVIIITVW	50
GPCR2	VDKRLQSPMYEFLSHISTLEILVTITIVPMMLWGLLELCROYLSLH---	96
AAK70859	VDKRLQSPMYEFLGNLSLLEILVTITIVPLMLWGLLPG-KOTISLNGCI	98
Q9Z1V0	VDRRLTPMYFPLRNFAMLEIWTSTVIEPKMLTN-TITG-HKTISLLGCF	96
Q9EPV0	VTGSLHKPMYYFLSIMSFLEAWYSVTVPKMLAGFLERP--NTISFLGCM	96
Q9EPG1	VTGSLHKPMYYFLSIMSFLEAWYSVTVPKMLAGFLHP--NTISFLGCM	96
Q9EPG2	STGSLHKPMYYFLSSMSFLEIWIYSVTVPKMLDGFLQR--RHISFTGCM	98
GPCR2	--VSLNFSCTMERALLGVMAVDRYVAVCNPLRYNTIMNSSTCIWVVIIS	144
AAK70859	AQLTLYLALGTTEFAVLGAMAVDRYVAVCNPLRYSVIMNRTCIWVVMVS	148
Q9Z1V0	LQARLYFFLTGTEFFLLAVMSFDRYVAICNPLRYATIMSKRVCVQLVFC	146
Q9EPV0	TQLYFFMSLACTECVLLAAMAYDRYVAICWPLRYPVIMTTGRCVQLTISS	146
Q9EPG1	TQLYFFMSLACTECVLLAAMAYDRYVAICWPLRYPVIMTTGRCVQLTISS	146
Q9EPG2	TQLYFFISLACTECVLLAAMAYDRYVAICWPLRYPVIMTTVYCMQLMALS	148
GPCR2	WVFGFLSEIWPVYATFQFTFRKSNLSLHFYCDRGQLLKLSCDNTLLTEFI	194
AAK70859	WVFGFLSEIWPVYATFQFTFKSNLSLHFYCDRGQLLKLSCNTIELTEFI	198
Q9Z1V0	WMSGLLIIVPSSIVFQPFCCPNINHHFCDNFPLMELICATSLVEFL	196
Q9EPV0	WVSGFTISMAYVYFISRVAFCGNNVLNHHFCDVSPILKLACMNLMAETV	196
Q9EPG1	WVSGFTISMAYVYFISRVAFCGNNVLNHHFCDVSPILKLACMNLMAETV	196
Q9EPG2	WVSGFMVSVVYVYFISHVAFCGSNVNHFFCDISPIKLACKDMSTAELV	198
GPCR2	LFLMAYFILIGSLIPTIVSYTYIISTILKIPASGRKKAFTFASHFTCV	244
AAK70859	LFLMAIFILVGSLLIPTIVSYTYIISTILKIPASGRKKAFTCASHFTFV	248
Q9Z1V0	GFVIANFSLIGLAVTATCYGHILYTLHIPSAKERKKAFTCSHIIIV	246
Q9EPV0	DFALAIVILIFPLSATVLSYGFIVSTVLOIPSATGORKAFTCASHLTVV	246
Q9EPG1	DFALAIVILIFPLSATVLSYGFIVSTVLOIPSATGORKAFTCASHLTVV	246
Q9EPG2	DFALAIVILIFPLITVLSYVYIVSTILIPSTQGRKKAFTCASHLTVV	248
GPCR2	VIGYGSCIFLYVKP-KOTQGVYENKIVSLVSVLTPPESTHLYSSDDKV	293
AAK70859	VIGYGTCLFLYVKP-KOTQAAEYNRVASLLVSVVTPFLNPFITLNRNDKV	297
Q9Z1V0	SLFYGSCIFMYVRSCKNGOGEDHNKVALLNIVVTPTLNPFIYTLRNKV	296
Q9EPV0	VIPYTAVIFMYVRP-RAIASFNSNKLISATYAVFTPMLNPITYCLRNKEV	295
Q9EPG1	VIPYTAVIFMYVRP-RAIASFNSNKLISATYAVFTPMLNPITYCLRNKEV	295
Q9EPG2	TIYTAIFMYVRP-RAIASFNSNKLISATYAVFTPMLNPITYCLRNREV	297
GPCR2	KEALRDGMKRCCQLLDK----	310
AAK70859	KEALRDGVKRCCLLRD----	314
Q9Z1V0	KQVEREHVSKFKFSOT----	313
Q9EPV0	KDAIRKTIAGGRAPALGESIS	316
Q9EPG1	KDAIRKTIAGGRAPALGESIS	316
Q9EPG2	KDAIRKTLGGGQCFLLC----	314

Table 2H lists the domain description from DOMAIN analysis results against GPCR2. This indicates that the GPCR2 sequence has properties similar to those of other proteins

known to contain this domain as well as to the 254 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 2H. Domain Analysis of GPCR2

PSSMs producing significant alignments:			Score (bits)	E value
7tm_1 (InterPro)	7 transmembrane receptor (rhodopsin family)		65.7	5.2e-20

5	7tm_1	1	*->GNLLVilvilrtkklrtptnifilNLAVADLLflltlppwalyylvg		47
			GN ++i++++ k+l++p+++f+ +L+ +L+++ +p++l l++		
	GPCR2	40	GNTVIIIVIVCVDKRLQSPMYFFLSHLSTLEILVTTIIVPMMLWGLLF		86
	7tm_1	48	gsedWpfGsalCklvtaldvvnmyaSillLtaISiDRYlAIvhPlryrrr		97
10			++ ++ l ++l + +++ lL ++++DRY+A+++Plry+ +		
	GPCR2	87	LGCRQYLS-----LHVSLNFSCGTMEFALLGVMAVDRYVAVCNPLRYNII		131
	7tm_1	98	rtsprrrAkvvillvWvlall	117	
			++ + v+++ Wv+++l		
15	GPCR2	132	MN-SSTCIWVVIWSWVFGFL	150	

The Olfactory Receptor-like GPCR2 disclosed in this invention is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR2 is provided in Example 2.

The nucleic acids and proteins of GPCR2 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further herein. The novel GPCR2 nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic

applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the “Anti-GPCRX Antibodies” section below. The disclosed GPCR2 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR2 epitope is from about amino acids 5 to 25. In additional embodiments, GPCR2 epitopes are from about amino acids 50 to 60, from about amino acids 80 to 100, from about amino acids 130 to 145, from about amino acids 230 to 240, from about amino acids 260 to 270 and from about amino acids 290 to 310.

GPCR3

The disclosed novel GPCR3 (alternatively referred to herein as sggc_draft_ba656o22_20000731_da4) includes the 971 nucleotide sequence (SEQ ID NO:27) shown in Table 5A. A GPCR3 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 3-5 and ends with a TAG codon at nucleotides 963-965. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 3A, and the start and stop codons are in bold letters.

Table 3A. GPCR3 Nucleotide Sequence (SEQ ID NO:27)

CAATGATGGAAATAGCCAATGTGAGTTCTCCAGAAGTCTTTGTCCTCCTGGGCTTCTCCGCACGACC
CTCACTAGAAACTGTCTCTTCATAGTTGTCTTGAGTTTACATGGTATCGATCTTGGGCAATGGC
ATCATCATTCTGGTCTCCCATACAGATGTGCACCTCCACACACCTATGTACTTCTTTCTTGCCAAACC
TCTCCTTCTGGACATGAGCTTCACCACGAGCATTGTCCACAGCTCCTGGCTAACCTCTGGGGACC
ACAGAAAACCATAAGCTATGGAGGGTGTGTGGTCCAGTTCTATATCTCCCATTTGGCTGGGGGCAACC
GAGTGTGTCCTGCTGGCCACCATGTCTATGACCGCTACGCTGCCATCTGCAGGCCACTCCATTACA
CTGTCATTATGCATCCACAGCTTTGCCCTTGGGCTAGCTTTGGCCTCCTGGCTGGGGGTCTGACCAC
CAGCATGGTGGGCTCCACGCTCACCATGCTCCTACCGCTGTGTGGGAACAATTGCATCGACCACTTC
TTTTGCGAGATGCCCCCTCATTATGCAACTGGCTTGTGTGGATACCAGCCTCAATGAGATGGAGATGT
ACCTGGCCAGCTTTGTCTTTGTTGTCTGCTCTGGGGCTCATCCTGGTCTCTTACGGCCACATTGC
CCGGGCCGTGTTGAAGATCAGGTGAGCAGAAGGGCGGAGAAAGGCATTCAACACCTGTTCTTCCCAC
GTGGCTGTGGTGTCTCTGTTTACGGGAGCATCATCTTCATGTATCTCCAGCCAGCCAAGAGCACCT
CCCATGAGCAGGGCAAGTTCATAGCTCTGTCTACACCGTAGTCACTCCTGCGTTGAACCCAGTTAT
TTACACCTGAGGAACACGGAGGTGAAGAGCGCCCTCCGGCACATGGTATTAGAGAACTGCTGTGGC
TCTGCAGGCAAGCTGGCGCAAATTTAGAGACTC

The GPCR3 polypeptide (SEQ ID NO:28) encoded by SEQ ID NO:27 is 320 amino acids in length, has a molecular weight of 35321.4 Daltons, and is presented using the one-letter amino acid code in Table 3B. The Psort profile for GPCR3 predicts that these sequences have a signal peptide and are likely to be localized at the endoplasmic reticulum (membrane) with a certainty of 0.6850. In alternative embodiments, a GPCR3 polypeptide is located to the plasma membrane with a certainty of 0.6400, the Golgi body with a certainty of 0.4600, or the endoplasmic reticulum (lumen) with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a GPCR3 peptide is between positions 44 and 45, *i.e.*, at the dash in the sequence GNG-II.

Table 3B. GPCR3 protein sequence (SEQ ID NO:28)

MMEIANVSSPEVFLVLLGFSARPSLETVLFIVVLSFYMVSIILNGIIILVSHTDVHLHTPMYFFLANLS
FLDMSFTTSIVPQLLANLWGPQKTISYGGCVVQFYISHWLGATECVLLATMSYDRYAAICRPLHYTVI
MHPQLCLGLALASWLGGLTTSVMVGSTLTMLLPLCGNNCIDHFFCEMPLIMQLACVDTSLNEMEMYLAS
FVFVVLPLGLILVSYGHIARAVLKIRSAEGRRKAFNTCSSHVAVVSLFYGSIIFMYLQPAKSTSHEQG
KFIALFYTVVTPALNPVIYTLRNTEVKSA LRHVMLENC CGSAGKLAQI

Additional SNP variants of GPCR3 are disclosed in Example 3. The amino acid sequence of GPCR3 had high homology to other proteins as shown in Table 3C.

Table 3C. BLASTX results for GPCR3

Sequences producing High-scoring Segment Pairs:	High Score	Prob P(N)	Smallest Sum
patp:AAG71896 Human olfactory receptor polypeptide 319 aa	1638	2.5e-168	
patp:AAG71912 Human olfactory receptor polypeptide 319 aa	1638	2.5e-168	
patp:AAG71891 Human olfactory receptor polypeptide 309 aa	983	6.5e-99	
patp:AAG72908 Human olfactory receptor 312 aa	982	8.3e-99	

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of this invention has 602 of 906 bases (66%) identical to a gb:GENBANK-ID:AF098664|acc:AF098664 mRNA from *Homo sapiens* [*Homo sapiens* olfactory receptor-like protein (OR2C1) gene, complete cds]. The full amino acid sequence of the protein of the invention was found to have 182 of 305 amino acid residues (59%) identical to, and 233 of 305 amino acid residues (76%) similar to, the 312 amino acid residue ptnr:SWISSPROT-ACC:P23275 protein from *Mus musculus* (Mouse) [OLFACTORY RECEPTOR 15 (OR3)].

Additional BLASTP results are shown in Table 3D.

Table 3D. GPCR3 BLASTP results

Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
P23275	OLFACTORY RECEPTOR 15 (OR3) - <i>Mus musculus</i> (Mouse)	312	182/305 (59%)	233/305 (76%)	1.3e-98
Q9GZK6	OLFACTORY RECEPTOR - <i>Homo sapiens</i> (Human)	312	176/306 (57%)	231/306 (75%)	1.1e-97
Q9GZK1	OLFACTORY RECEPTOR - <i>Homo sapiens</i> (Human)	312	176/306 (57%)	231/306 (75%)	1.8e-97
O76001	Olfactory receptor 2J3 (Olfactory receptor 6-6) (OR6-6) (Hs6M1-3) - <i>Homo sapiens</i> (Human)	311	174/301 (57%)	232/301 (77%)	3.0e-97
Q9Y3N9	Olfactory receptor 2W1 (Hs6M1-15) - <i>Homo sapiens</i> (Human)	320	173/305 (56%)	230/305 (75%)	1.0e-96

A multiple sequence alignment is given in Table 3E, with the GPCR3 protein of the invention being shown on line 1, in a ClustalW analysis comparing GPCR3 with related protein sequences disclosed in Table 3D.

Table 3E. Information for the ClustalW proteins:

1. SEQ ID NO:28, GPCR3
2. SEQ ID NO:29, P23275
3. SEQ ID NO:30, Q9GZK6
4. SEQ ID NO:31, Q9GZK1
5. SEQ ID NO:32, O76001
6. SEQ ID NO:33, Q9Y3N9

		10	20	30	40	50
15	GPCR3MMEIANVSSPEVFVLLGFSARPSLETIVLFIVVLSFYMVSIILGNGIIL			
	XX: P23275	---MEVDSNSSSGTFILMGVSDHPHLEIIFFAVILASYLLTLVGNLTIL				
	XX: Q9GZK6	--MLMKKNASFEDFILLGFSNWPHEVVLFFVILIFYLITLIGNLFIII				
	XX: Q9GZK1	--MLMKKNASFEDFELLGFSNWPHEVVLFFVILIFYLITLIGNLFIII				
20	XX: O76001	MNDGKVNASSEGIFILVGFSNWPHEVVLFFVILIFYLITLIGNLFIII				
	XX: Q9Y3N9	---MDQSNYSSLHGFILLGFSNHPKMEMILSGVVAIFYLITLVGNTAIL				
		60	70	80	90	100
25	GPCR3	VSHTDVHLHTPMYFFLANLSFLDMSFTTSIVPQLLANLWGPQKTISYGGC				
	XX: P23275	LSRLDARLHTPMYFFLSNLSSLDLAFTTSSVPQMLKNLWGPDKTISYGGC				
	XX: Q9GZK6	LSYLDShLHTPMYFFLSNLsFLDLcYTTSSIPQLLVNLWGPEKTISYAGC				
	XX: Q9GZK1	LSYLDShLHTPMYFFLSNLsFLDLcYTTSSIPQLLVNLWGPEKTISYAGC				
	XX: O76001	LSYLDShLHTPMYFFLSNLsFLDLcYTTSSIPQLLVNLWGPEKTISYAGC				
30	XX: Q9Y3N9	ASLLDSQLHTPMYFFLRNLsFLDLcFTTSIIPQLLVNLWGPDKTISYVGC				
		110	120	130	140	150
	GPCR3	VVQFYISHWLGCATECVLLATMSYDRAATCRPLHYTVIMHPQLCLGLALA				

5	XX: P23275	VTQLYVFLWLGATECILLVVMAFDRYVAVCRPLHYMTVMNPRLCWGLAAI
	XX: Q9GZK6	TVQLYFVLALGTAECVLLVVMMSYDRYAACVCRPLHYTVLMHPRFCRLAAAA
	XX: Q9GZK1	TVQLYFVLALGTAECVLLVVMMSYDRYAACVCRPLHYTVLMHPRFCRLAAAA
	XX: O76001	MTQLYFVLALGTTECVLLVVMMSYDRYAACVCRPLHYTVLMHPRFCHLLAVA
	XX: Q9Y3N9	IIQLYVVMWLGSVECLLLAVMSYDRFTAICKPLHYFVVMNPHLCLKMIIM
10	GPCR3
	XX: P23275	SWLGGLTTSVMVGSTLTLMLPLCGNNCIDHFFCEMPLIMQLACVDTSLNEM
	XX: Q9GZK6	SWLGGLGNSVIQSTFTLQIPFCGHRKVDNFLCEVPAMIKLACGDTSLNEA
	XX: Q9GZK1	SWVSGFTTSALHSSFTFWIPLCRHRLVDHFFCEVPALLRLSCVDTOANEL
	XX: O76001	SWVSGFTTSALHSSFTFWIPLCRHRLVDHFFCEVPALLRLSCVDTHVNEL
	XX: Q9Y3N9	IWSISLANSVVLCTLTNLPLTCGNNILDHFLCELPAIVKIAACVDTTTVM
15	GPCR3
	XX: P23275	EMYLASFVVFVVLPLGLILVSYGHIARAVLKIRSAEGRRKAFNTCSSHVAV
	XX: Q9GZK6	VLNGVCTFTTVVPVSVILVSYCFIAQAVMKIRSVGRRKAFNTCVSHLVV
	XX: Q9GZK1	TLMVMSSIFVLIPLILILITSYCAIARAVLSMQSTTCLQKVLRTCGAHLMV
	XX: O76001	TLMVMSSIFVLIPLILILITSYCAIARAVLSMQSTTCLQKVLRTCGAHLMV
	XX: Q9Y3N9	TLMITSSIFVLIPLILILITSYCAIARAVLRMQSTTCLQKVFGTCGAHLMA
20	GPCR3
	XX: P23275	VSLFYGSIIFMYLQPAKSTSHSQGKFIALFYTVVTPALNPVIYTLRNTEV
	XX: Q9GZK6	VSLFYGSAIYGYLLPAKSSNCSQGKFISLFYSVVTTPMVNPLIYTLRNKEV
	XX: Q9GZK1	VSLFFIPVMCMYLQPPSENSQDQGKFIALFYTVVTPSLNPLIYTFRNKDV
	XX: O76001	VSLFFIPVMCMYLQPPSENSQDQGKFIALFYTVVTPSLNPLIYTFRNKDV
	XX: Q9Y3N9	VSMFYGTIIYMYLQPGNRASKDQGKFILTIFYTVITPSLNPLIYTLRNKDM
25	GPCR3
	XX: P23275	VSLFYGSIIFMYLQPAKSTSHSQGKFIALFYTVVTPALNPVIYTLRNTEV
	XX: Q9GZK6	VSLFYGSAIYGYLLPAKSSNCSQGKFISLFYSVVTTPMVNPLIYTLRNKEV
	XX: Q9GZK1	VSLFFIPVMCMYLQPPSENSQDQGKFIALFYTVVTPSLNPLIYTFRNKDV
	XX: O76001	VSLFFIPAMCTYLOPPSGNSQDQGKFIALFYTVVTPSLNPLIYTLRNKVV
	XX: Q9Y3N9	VSMFYGTIIYMYLQPGNRASKDQGKFILTIFYTVITPSLNPLIYTLRNKDM
30	GPCR3
	XX: P23275	VSLFYGSIIFMYLQPAKSTSHSQGKFIALFYTVVTPALNPVIYTLRNTEV
	XX: Q9GZK6	VSLFYGSAIYGYLLPAKSSNCSQGKFISLFYSVVTTPMVNPLIYTLRNKEV
	XX: Q9GZK1	VSLFFIPVMCMYLQPPSENSQDQGKFIALFYTVVTPSLNPLIYTFRNKDV
	XX: O76001	VSLFFIPAMCTYLOPPSGNSQDQGKFIALFYTVVTPSLNPLIYTLRNKVV
	XX: Q9Y3N9	VSMFYGTIIYMYLQPGNRASKDQGKFILTIFYTVITPSLNPLIYTLRNKDM
35	GPCR3
	XX: P23275	KSALRHMVLENC CGSAGKLAQI-
	XX: Q9GZK6	KGALGRLLGKGRGAS-----
	XX: Q9GZK1	RGAVKRLMGWEWGM-----
	XX: O76001	RGAVKRLMGWE-----
	XX: Q9Y3N9	KDALKKLMRFHHKSTKIKRNCKS
40	GPCR3
	XX: P23275	KSALRHMVLENC CGSAGKLAQI-
	XX: Q9GZK6	KGALGRLLGKGRGAS-----
	XX: Q9GZK1	RGAVKRLMGWEWGM-----
	XX: O76001	RGAVKRLMGWE-----
	XX: Q9Y3N9	KDALKKLMRFHHKSTKIKRNCKS

Table 3F lists the domain description from DOMAIN analysis results against GPCR3. This protein contains domain IPR000276 at amino acid positions 42 to 291. This indicates that the GPCR3 sequence has properties similar to those of other proteins known to contain this domain as well as to the 254 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 3F Domain Analysis of GPCR3

PSSMs producing significant alignments:		Score (bits)	E value
gnl Pfam pfam00001	7tm_1, 7 transmembrane receptor (rhodopsin family)	144.2	9.9e-45

7tm_1 GNLLVILVILRTKKLRTPTNIFILNLAVADLLFLLTLPPWALYYLVG

5
 10
 15
 20
 25
 30
 35
 40

```

    GPCR3      42      ||+++|||      +|+||+++|+ ||++ |+ |+++ +| | +|+|
                      GNGIILVSHSTDVHLHTPMYFFLANLSFLDMSFTTSIVPQLLANLWG 88

    GPCR3      89      GSEDWPFGSALCKLVTALDVVNMYASILLTASIDRYLAIVHPLRYRRR
                      ++ +++ +| ++ ++ + + + + ||+ +|+||| ||++|+| ++
    GPCR3      89      --PQKTISYGGCVVQFYISHWLGATECVLLATMSYDRYAAICRPLHYTVI 136

    GPCR3      137     RTSPRRAKVVILLVWVLALLLSLPPLLFSWVKTVEEGNGTLNVNVTVCLI
                      + |+++++|+ | + +| | + ++ ++ ++ +||+ +++++| +
    GPCR3      137     MH-PQLCLGLALASWLGGLTTSVMVGSTL-TMLPLCGNNC--IDHFFCEM 182

    GPCR3      183     DFPEESTAS.VSTWLRSYVLLSTLVGFLLPLLVLVCYTRILRTLRL...
                      + ++ ++|+ ++ ++| |++++ +||| +||| |++|+|++ + ++
    GPCR3      183     PLIMQLACVDTSLNEMEMYLASFVFV-VLPLGLILVSYGHIARAVLKIRS 231

    GPCR3      232     ...KAAKTLVVVVVFLCWLPYFIVLLLDTLCL.SIIMSSTCELERVL
                      +++++ |+ ++ +|++| +++ |+++|++ +++| +
    GPCR3      232     AEGRRKAFNTCSSHVAVVSLFYGSIIFMYLQPAKSTS-----H 269

    GPCR3      270     PTALLVTLWLAYVNSCLNPIIY
                      + +++++|++++| ++|||+||
    GPCR3      270     EQGKFIALFYTVVTPALNPVIY 291
  
```

A GPCR-like protein of the invention, referred to herein as GPCR3, is an Olfactory Receptor ("OR")-like protein. Some members of the Olfactory Receptor-Like Protein Family end up localized at the cell surface, where they exhibit activity. Therefore it is likely that these novel GPCR3 proteins are available at the appropriate sub-cellular localization and hence accessible for the therapeutic uses described in this application.

The GPCR3 disclosed in this invention is expressed in at least some of the following tissues: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain - hippocampus, brain - substantia nigra, brain - thalamus, brain -whole, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. This information was derived by determining the tissue sources of the sequences that were included in the invention. Further expression data for GPCR3 is provided in Example 2.

The nucleic acids and proteins of GPCR3 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further above.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of

the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the “Anti-GPCRX Antibodies” section below. The disclosed GPCR3 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR3 epitope is from about amino acids 220 to 245. In another embodiment, a GPCR3 epitope is from about amino acids 255 to 270. In further specific embodiments, GPCR3 epitopes are from about amino acids 275 to 315.

GPCR4

A fourth GPCR-like protein of the invention, referred to herein as GPCR4, is an Olfactory Receptor (“OR”)-like protein. Some members of the Olfactory Receptor-Like Protein Family end up localized at the cell surface, where they exhibit activity. Therefore it is likely that these novel GPCR4 proteins are available at the appropriate sub-cellular localization and hence accessible for the therapeutic uses described in this application.

Two alternative novel GPCR4 nucleic acids and encoded polypeptides are provided, namely GPCR4a and GPCR4b.

GPCR4a

In one embodiment, a GPCR4 variant is the novel GPCR4a (alternatively referred to herein as AC0170103_A_da1), which includes the 1025 nucleotide sequence (SEQ ID NO:34) shown in Table 4A. A GPCR4a ORF begins with a Kozak consensus ATG initiation codon at nucleotides 33-35 and ends with a TGA codon at nucleotides 969-971. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4A, and the start and stop codons are in bold letters.

Table 4A. GPCR4a Nucleotide Sequence (SEQ ID NO:34)

```

AGCTGTGGACCATCTCTCAGAACTCTGCAGCATGGAGCCGCTCAACAGAACAGAGGTGTCCGAGTT
CTTTCTGAAAGGATTTTCTGGCTACCCAGCCCTGGAGCATCTGCTCTTCCCTCTGTGCTCAGCCATG
TACCTGGTGACCCTCCTGGGGAACACAGCCATCATGGCGGTGAGCGTGCTAGATATCCACCTGCACA
CGCCCGTGTACTTCTTCTGGGCAACCTCTCTACCCTGGACATCTGCTACACGCCCACCTTTGTGCC
TCTGATGCTGGTCCACCTCCTGTCTATCCCGGAAGACCATCTCCTTTGCTGTCTGTGCCATCCAGATG
TGTCTGAGCCTGTCCACGGGCTCCACGGAGTGCCTGCTACTGGCCATCACGGCCTATGACCGCTACC
TGGCCATCTGCCAGCCACTCAGGTACACGTGCTCATGAGCCACCGGCTCTGCGTGCTGCTGATGGG
AGCTGCCTGGGTCTCTGCTCCTCAAGTCGGTGACTGAGATGGTCATCTCCATGAGGCTGCCCTTC
TGTGGCCACCACGTGGTCAGTCACTTACCTGCAAGATCCTGGCAGTGCTGAAGCTGGCATGCGGCA

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ACACGTCGGTCAGCGAAGACTTCCTGCTGGCGGGCTCCATCCTGCTGCTGCCTGTACCCCTGGCATT
CATCTGCCTGTCTTACTTGCTCATCTGGCCACCATCCTGAGGGTGCCCTCGGCCGCCAGGTGCTGC
AAAGCCTTCTCCACCTGCTTGGCACACCTGGCTGTAGTGCTGCTTTTCTACGGCACCATCATCTTCA
TGTACTTGAAGCCCAAGAGTAAGGAAGCCCACATCTCTGATGAGGTCTTCACAGTCCTCTATGCCAT
GGTCACGACCATGCTGAACCCACCATCTACAGCCTGAGGAACAAGGAGGTGAAGGAGGCCGCCAGG
AAGGTGTGGGGCAGGAGTCGGGCCTCCAGGTGAGGGAGGGCGGGGCTCTGTACAGACGCAGGTCTCA
GGTTAGTAGCTGAGGCCATC

The sequence of GPCR4a was derived by laboratory cloning of cDNA fragments, by *in silico* prediction of the sequence. The cDNA fragments covering either the full length of the DNA sequence, or part of the sequence, or both, were cloned. *In silico* prediction was based on sequences available in CuraGen's proprietary sequence databases or in the public human sequence databases, and provided either the full length DNA sequence, or some portion thereof.

The cDNA coding for the GPCR4a sequence was cloned by the polymerase chain reaction (PCR). Primers were designed based on *in silico* predictions of the full length or some portion (one or more exons) of the cDNA/protein sequence of the invention. The DNA sequence and protein sequence for a novel Olfactory Receptor-like gene were obtained by exon linking and are reported here as GPCR4a. These primers and methods used to amplify GPCR4a cDNA are described in the Examples.

The GPCR4a polypeptide (SEQ ID NO:35) encoded by SEQ ID NO:34 is 312 amino acids in length, has a molecular weight of 34688.2 Daltons, and is presented using the one-letter amino acid code in Table 4B. The Psort profile for GPCR4a predicts that this sequence has a signal peptide and are likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, a GPCR4a polypeptide is located to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or a microbody (peroxisome) with a certainty of 0.3000. The Signal P predicts a likely cleavage site for a GPCR4a peptide is between positions 47 and 48, *i.e.*, at the dash in the sequence IMA-VS.

Table 4B. GPCR4a protein sequence (SEQ ID NO:35)

MEPLNRTEVSEFFLKGFSGYPALHLLFPLCSAMYLVTLLGNTAIMAVSVLDIHLHTPVYFFLGNLS
TLDICYPTTFVPLMLVHLLSSRKTISFAVCAIQMCLSLSTGSTECLLLAITAYDRYLAIQPLRYHV
LMSHRLCVLLMGAAWLCLKSVTEMVISMRLPFCGHVVSHTCKILAVLKLACGNTSVSEDFLLA
GSILLPVPLAFICLSYLLILATILRVPSAARCKAFSTCLAHLAVVLLFYGTIIIFYLKPKSKEAH
ISDEVFTVLYAMVTTMLNPTIYSLRNKEVKEARKVWGRSRASR

Additional SNP variants of GPCR4a are disclosed in Example 3.

GPCR4b

In an alternative embodiment, a GPCR4 variant is the novel GPCR4b (alternatively referred to herein as CG54212-03), which includes the 917 nucleotide sequence (SEQ ID NO:36) shown in Table 4C. The GPCR4b ORF was identified at nucleotides 3-5 with a CTC codon and ends with a TGA codon at nucleotides 861-863. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4C, and the start and stop codons are in bold letters.

Table 4C. GPCR4b Nucleotide Sequence (SEQ ID NO:36)

TGCTCTTCCCTCTGTGCTCAGCCATGTACCTGGTGACCTCCTGGGGAACACAGCCATCATGGCGGT
GAGCGTGCTAGATATCCACCTGCACACGCGCGTGTACTTCTTCTGGGCAACCTCTCTACCTGGAC
ATCTGCTACACGCCCACCTTTGTGCCTCTGATGCTGGTCCACCTCCTGTATCCCGGAAGACCATCT
CCTTTGCTGTCTGTGCCATCCAGATGTGTCTGAGCCTGTCCACGGGCTCCACGGAGTGCCTGCTACT
GGCCATCACGGCCTATGACCGCTACCTGGCCATCTGCCAGCCACTCAGGTACCACGTGCTCATGAGC
CACCGGCTCTGCGTGCTGCTGATGGGAGCTGCCTGGGTCTCTGCCTCCTCAAGTCGGTGACTGAGA
TGGTCATCTCCATGAGGCTGCCCTTCTGTGGCCACCACGTGGTCAGTCACTTCACCTGCAAGATCCT
GGCAGTGCTGAAGCTGGCATGCGGCAACACGTGCGTCAGCGAAGACTTCTGCTGGCGGGCTCCATC
CTGCTGCTGCCTGTACCCCTGGCATTCATCTGCCTGTCTACTTGGTCATCTGGCCACCATCCTGA
GGGTGCCCTCGGCCGCCAGGTGCTGCAAAGCCTTCTCCACCTGCTTGGCACACCTGGCTGTAGTGCT
GCTTTTCTACGGCACCATCATCTTCATGTACTTGAAGCCCAAGAGTAAGGAAGCCCACATCTCTGAT
GAGGTCTTCACAGTCTCTATGCCATGGTCACGACCATGCTGAACCCACCATCTACAGCCTGAGGA
ACAAGGAGGTGAAGGAGGCCGCCAGGAAGGTGTGGGCGAGGAGTGGGCCTCCAGGTGAGGGAGGGC
GGGGCTCTGTACAGACGCAGGTCTCAGGTTAGTAGCTGAGGCCATC

The GPCR4b protein (SEQ ID NO:37) encoded by SEQ ID NO:36 is 286 amino acids in length, has a molecular weight of 31693.8 Daltons, and is presented using the one-letter code in Table 4D. The Psort profile for GPCR4b predicts that this sequence has a signal peptide and is likely to be localized at the endoplasmic reticulum (membrane) with a certainty of 0.6850. In alternative embodiments, a GPCR4b polypeptide is located to the plasma membrane with a certainty of 0.6400, the Golgi body with a certainty of 0.4600, or to the endoplasmic reticulum (lumen) with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a GPCR4a peptide is between positions 21 and 22, *i.e.*, at the dash in the sequence IMA-VS.

Table 4D. GPCR4b protein sequence (SEQ ID NO:37)

LFPLCSAMYLVTLLGNTAIMAVSVLDIHLHTPVYFFLGNLSTLDICYTPTFVPLMLVHLLSSRKTIISFAVC
AIQMCLSLSTGSTECLELLAITAYDRYLAIQPLRYHVLMSHRLCVLLMGAAWVLCLLKSVTMVISMRLPF
CGHHVVSHTCKILAVLKLACGNTSVSEDFLLAGSILLPVPLAFICLSYLLILATILRVPSAARCKAFS
TCLAH LAVVLLFYGTIIIFMYLKPKSKEAHSDEVFTVLYAMVTTMLNPTIYSLRNKEVKEAARKVWGRSRA
SR

Additional SNP variants of GPCR4b are disclosed in Example 3.

GPCR4 Clones

Unless specifically addressed as GPCR4a or GPCR4b, any reference to GPCR4 is assumed to encompass all variants. Residue differences between any GPCR4 variant sequences herein are written to show the residue in the "a" variant, the residue position with respect to the "a" variant, and the residue in the "b" variant.

The amino acid sequence of GPCR4 has high homology to other proteins as shown in Table 4E.

Table 4E. BLASTX results for GPCR4			
		High	Smallest
		Score	Sum
Sequences producing High-scoring Segment Pairs:			Prob
			P (N)
patp:AAG71899	Human olfactory receptor polypeptide, 312 aa	1603	1.3e-164
patp:AAG71954	Human olfactory receptor polypeptide, 333 aa	883	2.6e-98
patp:AAG71946	Human olfactory receptor polypeptide, 316 aa	848	1.3e-84

In a search of sequence databases, it was found, for example, that the GPCR4a nucleic acid sequence has 852 of 1010 bases (84 %) identical to a *Mus musculus* or17c gene mRNA (GENBANK-ID: MMU133429|acc:AJ133429). The full amino acid sequence of the GPCR4a protein of the invention was found to have 256 of 312 amino acid residues (82%) identical to, and 275 of 312 residues (88 %) positive with, the 317 amino acid residue OLFACTORY RECEPTOR-LIKE PROTEIN protein from *Mus musculus*, 312 aa (ptnr: SPTREMBL-ACC:Q9QZ18).

Similarly, in a search of sequence databases, it was found, for example, that the GPCR4b nucleic acid sequence of this invention has 781 of 920 bases (84%) identical to a gb:GENBANK-ID:MMU133429|acc:AJ133429.1 mRNA from *Mus musculus* (*Mus musculus* or17 gene). The full amino acid sequence of the GPCR4b protein of the invention was found to have 234 of 286 amino acid residues (81%) identical to, and 253 of 286 amino acid residues (88%) similar to, the 312 amino acid residue ptnr:SPTREMBL-ACC:Q9QZ18 protein from *Mus musculus* (Mouse) (OLFACTORY RECEPTOR).

Additional BLASTP results are shown in Table 4F.

GPCR4 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Q9QZ18	OLFACTORY RECEPTOR - <i>Mus musculus</i> (Mouse)	312	256/312 (82%)	275/312 (88%)	1.2e-134
AAK95088	OLFACTORY RECEPTOR - <i>Homo sapiens</i> (Human)	216	216/216 (100%)	216/216 (100%)	4.4e-112
Q9QZ22	OLFACTORY RECEPTOR - <i>Mus Musculus</i> (Mouse)	319	166/314 (52%)	224/314 (71%)	2.1e-82
Q9QZ19	OLFACTORY RECEPTOR - <i>Mus Musculus</i> (Mouse)	319	167/314 (53%)	221/314 (70%)	2.1e-82
Q9QZ21	OLFACTORY RECEPTOR - <i>Mus Musculus</i> (Mouse)	318	164/313 (52%)	226/313 (72%)	3.4e-82

A multiple sequence alignment is given in Table 4G, with the GPCR4 protein of the invention being shown on line 1 and 2, in a ClustalW analysis comparing GPCR4 with related protein sequences of Table 4F. The residue that differs between GPCR4a and GPCR4b is marked with the (o) symbol.

Table 4G. Information for the ClustalW proteins:

1. SEQ ID NO:35, GPCR4a
2. SEQ ID NO:37, GPCR4b
3. SEQ ID NO:38, Q9QZ18
4. SEQ ID NO:39, AAK95088
5. SEQ ID NO:40, Q9QZ22
6. SEQ ID NO:41, Q9QZ19
7. SEQ ID NO:42, Q9QZ21

		10	20	30	40	50
GPCR4a	MEPLN	RTE-VSEFF	LKCFSGYPALEHLLRPLCSAMYLVTLLGNTAIMAVS			
GPCR4b	MEPLN	RTE-VSEFF	LKCFSGYPALEHLLRPLCSAMYLVTLLGNTAIMAVS			
XX: Q9QZ18	MEPSN	RTA-VSEFV	LKCFSGYPALEHLLRPLCSAMYLVTLLGNTAIMAVS			
XX: AAK950						
XX: Q9QZ22	MDRSE	NETAPLSGFI	LLGLSAHPKLEKTFEVLILMYLVILLGNGVLILVS			
XX: Q9QZ19	MERSN	KTTVPVSSFI	LLGLSAHPKLEKTFEVLILMYLVILLGNGVLILVS			
XX: Q9QZ21	MEGAN	QST-VAEFV	LLGLSDHPKLEKTFEVLILMYLVILLGNGVLILVS			
		60	70	80	90	100
GPCR4a	VLDIHLHTP	MYFFLGNLS	TLDICYTPTFVPLMLVHLLSSRKTISFAVCAI			
GPCR4b	VLDIHLHTP	MYFFLGNLS	TLDICYTPTFVPLMLVHLLSSRKTISFAVCAI			
XX: Q9QZ18	MLDARLHTP	MYFFLGNLS	TLDICYTPTFVPLMLVHLLSSRKTISFAVCAI			
XX: AAK950						
XX: Q9QZ22	ILD	SHLHTP	MYFFLGNLS	TLDICYTPTFVPLMLVHLLSSRKTISFAVCAI		
XX: Q9QZ19	ILD	SHLHTP	MYFFLGNLS	TLDICYTPTFVPLMLVHLLSSRKTISFAVCAI		
XX: Q9QZ21	ILD	SHLHTP	MYFFLGNLS	TLDICYTPTFVPLMLVHLLSSRKTISFAVCAI		
		110	120	130	140	150
GPCR4a	QMCLSLSTG	STECCLLAITAY	DRYLAICOP	PLRYHVLMSHRLCVLLMGAAW		
GPCR4b	QMCLSLSTG	STECCLLAITAY	DRYLAICOP	PLRYHVLMSHRLCVLLMGAAW		
XX: Q9QZ18	QMCLSLSTG	STECCLLAITAY	DRYLAICOP	PLRYHVLMSHRLCVLLMGAAW		
XX: AAK950	QMCLSLSTG	STECCLLAITAY	DRYLAICOP	PLRYHVLMSHRLCVLLMGAAW		
XX: Q9QZ22	QMFLSFAMG	ATECVLLSMM	AFDRYVAICN	PLRYHVLMSHRLCVLLMGAAW		

XX: Q9QZ19 QMFLSFAMGATECVLLGMMAFDRYVLAICNPLRYPVVMSKAAYVPMAGSW
 XX: Q9QZ21 QMFLSFAMGATECVLLGMMAFDRYVLAICNPLRYPVVVNKSAYVPMVSSW

		160	170	180	190	200
5					
	GPCR4a	VLCLLKSVTEMVISMRLPFCGHHVVSHTFCKILAVLKLACGNTSVSEDFL				
	GPCR4b	VLCLLKSVTEMVISMRLPFCGHHVVSHTFCKILAVLKLACGNTSVSEDFL				
	XX: Q9QZ18	VLCLFKSVAETVIAMRLPFCGHHVIRHFTCEILAVLKLTCGDTSVSDAFL				
	XX: AAK950	VLCLLKSVTEMVISMRLPFCGHHVVSHTFCKILAVLKLACGNTSVSEDFL				
10					
	XX: Q9QZ22	AGGITNSVQTSIAMRLPFCGDNVINHFTCEILAVLKLACADISINVISM				
	XX: Q9QZ19	VSGSITATVQISLAMTLPCGDNVINHFTCEILAVLKLACADISINVISM				
	XX: Q9QZ21	VAGGANSLVQISLAVQLPCGDNVINHFTCEILAVLKLACADISINVISM				
		210	220	230	240	250
15					
	GPCR4a	LAGSILLIPVPLAFICLSYLLILATILRVPSAARCKAFSTCLAHLAHVVL				
	GPCR4b	LAGSILLIPVPLAFICLSYLLILATILRVPSAARCKAFSTCLAHLAHVVL				
	XX: Q9QZ18	LVGAILLPVPLTLICLSYMLILATILRVPSATGRSKAFSTCSAHLAVVL				
	XX: AAK950	LAGSILLIPVPLAFICLSYLLILATILRVPSAARCKAFSTCLAHLAHVVL				
20					
	XX: Q9QZ22	VVANMIFLAVPVLFIFVSYVFIILVTILRIPSAEGRKKAFSTCSAHLTVVL				
	XX: Q9QZ19	AVANAMFLGVPVLFIFVSYIFILSTILRIPSAEGRKKAFSTCSAHLTVVL				
	XX: Q9QZ21	GVANVIFLGVVLFIFVSYIFILSTILRIPSAEGRKKAFSTCSAHLTVVL				
		260	270	280	290	300
25					
	GPCR4a	LFYGTIIIFMYLKPKSKE-----AHISDEVFTVLVAMVTMLNPTIYSLR				
	GPCR4b	LFYGTIIIFMYLKPKSKE-----AHISDEVFTVLVAMVTMLNPTIYSLR				
	XX: Q9QZ18	LFYGTIIIFMYMKPKSKE-----ARISDQVFTVLVAVVTMLNPTIYSLR				
	XX: AAK950	LFYGTIIIFMYLKPKSKE-----AHISDEVFTVLVAMVT-----				
30					
	XX: Q9QZ22	VFYGTILFMYGKPKSKDPLGADKQDLADKLISLFYGVVTPMLNPTIYSLR				
	XX: Q9QZ19	VFYGTILFMYGKPKSKDPLGADKQDLADKLISLFYGVVTPMLNPTIYSLR				
	XX: Q9QZ21	VFYGTILFMYGKPKSKDPLGADKQDVSDKLISLFYGVLTMLNPTIYSLR				
		310				
35					
	GPCR4a	NKEVKEAARKVWGRSRASR				
	GPCR4b	NKEVKEAARKVWGRSRASR				
	XX: Q9QZ18	NKEVKEAARKAWGSRWACR				
	XX: AAK950	-----				
40					
	XX: Q9QZ22	NKDVRAAARNLVGQKHLTE				
	XX: Q9QZ19	NKDVRAAAVTNLVGQKHFKW				
	XX: Q9QZ21	NKDVRAAARNLVGQKCLIQ				

45 DOMAIN results for GPCR4 were collected from the Conserved Domain Database
 (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the
 Smart and Pfam collections. The results are listed in Table 4H with the statistics and domain
 description. The 7tm_1, a seven transmembrane receptor (rhodopsin family), was shown to
 have significant homology to GPCR4. An alignment of GPCR4b residues 40-290 (SEQ ID
 50 NO:X) with 7tm_1 residues 1-254 (SEQ ID NO:18) are shown in Table 4H.

Table 4H. DOMAIN results for GPCR4

PSSMs producing significant alignments:

	Score	E
	(bits)	value
gnl Pfam pfam00001 7tm_1, 7 transmembrane receptor (rhodopsin family)	123.1	4.6e-38

7tm_1 GNLLVILVILRTKKLRTPTNIFILNLAVADLLFLLTLPPWALYYLVG
 || +++| ++ +|+|| ++|++||+ |++++ | +| +|++|+
 5 GPCR4 41 GNTAIMAVSVLDIHLHTPVYFVLGNLSTLDICYTPTFVPLMLVHLLS 87

GSSEDWPFSGSALCKLVLTALDVVNMYASILLTALSIDRYLAIVHPLRYRRR
 +++ ++ |+| +++++| + ++ + +|||+ ++| |||||+ ||||+++
 10 GPCR4 88 --SRKTISFAVCAIQMCLSLSTGSTECLLLTAITAYDRYLAICQPLRYHVL 135

RTSPRRAKVVILLVWVLALLLSLPLLFVSWVKTVEEGNGTLNVNVTVCLI
 ++ +|+++++ +++|||+|| |+ + +++++++|+++ |+++| |
 GPCR4 136 MS-HRLCVLLMGAAWVLCLLKSVTE-MVISMRLPFCGHHV--VSHFTCKI 181

DFPEESTASVSTWLRSYVLLSTLVGFLLPLLVLVCYTRILRTLRL....
 + +++ +++++ + | +++ +|| +|+ |++|| |+ + ++
 15 GPCR4 182 LAVLKLACGNTSVSEDFLLAGSILLLPVPLAFICLSYLLILATILRVPSA 231

...KAAKTLVVVVVFLCWLPHYFIVLLLDTLCLSIIMSSTCELERVLP
 + |+ +++ +++++|++++ |+++| + +++ +
 20 GPCR4 232 ARCKAFSTCLAHLAVVLLFYGTIIIFYLKPKSKEA-----HI 269

TALLVTLWLAYVNSCLNPIIY
 +|+++|+| +||| ||
 25 GPCR4 270 SDEVFTVLYAMVTTMLNPTIY 290

The cDNA coding for the GPCR4b sequence was cloned by the polymerase chain reaction (PCR). Primers were designed based on *in silico* predictions of the full length or some portion (one or more exons) of the cDNA/protein sequence of the invention. The DNA sequence and protein sequence for a novel Olfactory Receptor-like gene were obtained by exon linking and are reported here as GPCR4b. These primers and methods used to amplify GPCR4b cDNA are described in the Examples.

The GPCR4 disclosed in this invention is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to

SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. This is by no way limiting in that olfactory receptors are a class of G protein-coupled receptor which are known to be expressed in all tissue types. Further tissue expression analysis is provided in Example 2.

The nucleic acids and proteins of GPCR4 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further above.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR_X Antibodies" section below. The disclosed GPCR4b protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR4b epitope is from about amino acids 220 to 250. In other specific embodiments, GPCR4b epitopes are from about amino acids 255 to 290.

GPCR5

The disclosed novel GPCR5 (alternatively referred to herein as 21629632.0.20) includes the 2028 nucleotide sequence (SEQ ID NO:43) shown in Table 5A. A GPCR5 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 469-471 and ends with a TAG codon at nucleotides 1447-1449. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 5A, and the start and stop codons are in bold letters.

Table 5A. GPCR5 Nucleotide Sequence (SEQ ID NO:43)

<p>TGCTATAGCCCCAGCACTTGATACCTAGCACAGAATAGGTACTTAATAAATACTTAGTGGATGAATAAATCTG AAATACTATGGCCATAATTTGGTCACATGAAGCCGTAATGTAGAAAAGATGCTTCCTGTTAATGACCAAAAAC ACTTTGGATTCCAAACGATCATTTTAAACATGAATCTTTCTGCTGTCTCCTCTGACCCCATCCTGGGGAGA GCAGAGAGGAGCCTAGGGGACTAGAATGTGCCCCATCCTCCCCTCAGTGACGTCCACAGAACTGCAGCGCTGA GAAGGCCAGATTGCAGATCTGAAGTCCAACCTCCCTCATTATACAGATGGTGAAACTAAATTCAGAGAGGGAG GCTGACCTGCTGCAGCTCAGACATCAGGTCACTGGGCTCCCAGGCCAGTTGGAGCTTTTCCAAAAAGCTGGG TGGTCCAGATGGAAAAGGAGAGAGAATGAGATGAAGTGGGCAAACAGACAGCTGTGACGGAATACGTCTCTGA TGGGGCTACACGAGCACTGTAACCTGGAGGTGCTCCTGTTTGTGTTCTGCCTGGGCATCTACTCCGTGAATGT GTTGGGGAACGCCCTCCTCATAGGGCTGAACGTGCTGCACCCTCGCCTGCACAACCCCATGTACTTCCTTCTC</p>

AGCAACCTCTCCCTCATGGACATCTGCGGCACCTCCTCCTTTGTGCCTCTCATGCTAGACAATTTCTGGAAA
 CCCAGAGGACCAATTTCCCTTCCCTGGCTGTGCCCTGCAGATGTACCTGACCCTGGCGCTGGGATCAACGGAGTG
 CCTGCTGCTGGCTGTGATGGCATATGACCGTTATGTGGCTATCTGCCAGCCGCTTAGGTACCCAGAGCTCATG
 AGTGGGCGAGACCTGCATGCAGATGGCAGCGCTGAGCTGGGGGACAGGCTTTGCCAACTCACTGCTACAGTCCA
 TCCTTGTCTGGCACCTCCCCTTCTGTGGCCACGTCACTACTTCTATGAGATCTTGGCAGTGCTAAAACT
 GGCTGTGGGGACATCTCCCTCAATGCGCTGGCATTAAATGGTGGCCACAGCCGCTCCTGACACTGGCCCCCTC
 TTGCTCATCTGCCTGTCTTACCTTTTTCATCCTGTCTGCCATCCTTAGGGTACCCTCTGCTGCAGGCCGGTGCA
 AAGCCTTCTCCACCTGCTCAGCCACCGCACAGTGGTGGTGGTTTTTATGGGACAATCTCCTTCATGTACTT
 CAAACCCAAGGCCAAGGATCCCAACGTGGATAAGACTGTGCGATTGTTCTACGGGGTTGTGACGCCCTCGCTG
 AACCCCATCATTTACAGCCTGAGGAATGCAGAGGTGAAAGCTGCCGTCTAACTCTGCTGAGAGGAGGTTTGC
 TCTCCAGGAAAGCATCCCACTGCTACTGCTGCCCTCTGCCCCGTGCTGAGTGGCATAGGCTAGGTTGTGCTGTG
 GTCATGACCTCAAACCTTGAGAGGCTTAAAGCCATTAAGGTTTGTTCCTTGCTCCTGATGCAGGTCCACCAGA
 GGCTGGTGGGGCTTCTGCTCCGCATCATGGTCTTCAACCCCTCTGGGACTCAGGATGACAAAACAGCTACCATT
 GGGAACTGCTGGTCAACCATGACAAAAAGAAAGGGAAAGTAACAAAGCCTACACTGACTCTTAAAGCTTCT
 ACTCAGAAGTGGCTGTGTTGCCTCCACCTACATTTCACTGGCCAAACAATGGCAACAGGAAGGCACAGGACC
 ACACCTATTGTTAAGGGGGAAAAGCACACTATCGTGTGTCTGGATGGCAAACGAGAGGGACAGAGAGATTTGT
 GAATGGCCTAATGACTACCACACAGCTGACAGTGTCAACCCAAGAGCTATGGGAGGTTTGGCTTTCTTTATC
 CTGACCATCTATCCTTCACGGGCTGCTGCCAAGTTAATCGTCCCAAGAAAGCTCTGGTTAGCTCACGTGTGGT
 AGCTTTTACTAGTCAACCAAAGTAGGCTAGAGGGTGTGGGTTAGGGTTGGCCACA

The GPCR5 polypeptide (SEQ ID NO:44) encoded by SEQ ID NO:43 is 326 amino acids in length, has a molecular weight of 35715.1 Daltons, and is presented using the one-letter amino acid code in Table 5B. The Psort profile for GPCR5 predicts that these sequences have a signal peptide and are likely to be localized at the plasma membrane with a certainty of 0.0.6000. In alternative embodiments, a GPCR5 polypeptide is located to the Golgi body with a certainty of 0.0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the mitochondrial inner membrane with a certainty of 0.3000.

Table 5B. GPCR5 protein sequence (SEQ ID NO:44)

MKWANQTAVTEYVLMGLHEHCNLEVVLFVFLGLIYSVNVLGNALLIGLNVLHPRLHNPMYFLLSNLS
 LMDICGTSSFVPLMLDNFLETQRTISFPGCALQMYLTLALGSTECLLAVMAYDRYVAICQPLRYPE
 LMSGQTCMQMAALSWGTFANSLLSILVWHLFPFGHVINYFYBILAVLKLACGDISLNALALMVAT
 AVLTLAPLLLLICLSYLFILSAILRVPSAAGRCKAFSTCSAHRVTVVVVFGTISFMYFKPKAKDPNVD
 KTVALFYGVVTPSLNPIIYSLRNAEVKAAVLTLLRGGLLSRKASHCYCCPLPLSAGIG

Additional SNP variants of GPCR5 are disclosed in Example 3. The amino acid sequence of GPCR5 had high homology to other proteins as shown in Table 5C.

Table 5C. BLASTX results for GPCR5

Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Prob P (N)	
		High Score	Smallest Sum Prob P (N)
patp:AAG71895 Human olfactory receptor polypeptide, 311 aa	1588	5.2e-163	
patp:AAG71924 Human olfactory receptor polypeptide, 197 aa	987	2.5e-99	
patp:AAG72651 Murine OR-like polypeptide, 356 aa	895	1.4e-89	

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of this invention has 1024 of 1351 bases (75%) identical to a gb:GENBANK-ID:MMU133430|acc:AJ133430 mRNA from *Mus musculus* (*Mus musculus* or6 gene, Fig. 3A). The full amino acid sequence of the protein of the invention was found to have 230 of 314 amino acid residues (73%) identical to, and 261 of 314 amino acid residues (83%) similar to, the 315 amino acid residue ptnr:SPTREMBL-ACC:Q9QZ17 protein from *Mus musculus* (Mouse) (OLFACTORY RECEPTOR).

Additional BLASTP results are shown in Table 5D.

Table 5D. GPCR5 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Q9QZ17	OLFACTORY RECEPTOR - <i>Mus musculus</i> (Mouse)	315	230/314 (73%)	261/314 (83%)	1.9e-118
Q9QZ20	OLFACTORY RECEPTOR - <i>Mus musculus</i> (Mouse)	318	182/310 (58%)	227/310 (73%)	2.1e-89
Q9QZ21	OLFACTORY RECEPTOR - <i>Mus musculus</i> (Mouse)	318	182/311 (58%)	231/311 (74%)	9.0e-89
Q9QZ22	OLFACTORY RECEPTOR - <i>Mus musculus</i> (Mouse)	318	178/312 (57%)	231/312 (74%)	1.3e-87
Q9QZ19	OLFACTORY RECEPTOR - <i>Mus musculus</i> (Mouse)	319	180/312 (57%)	229/312 (73%)	1.2e-86

A multiple sequence alignment is given in Table 5E, with the GPCR5 protein of the invention being shown on line 1, in a ClustalW analysis comparing GPCR5 with related protein sequences disclosed in Table 5D.

Table 5E. Information for the ClustalW proteins:

1. SEQ ID NO:44, GPCR5
2. SEQ ID NO:45, Q9QZ17, Olfactory receptor- *Mus musculus*
3. SEQ ID NO:46, Q9QZ20, Olfactory receptor- *Mus musculus*
4. SEQ ID NO:47, Q9QZ21, Olfactory receptor- *Mus musculus*
5. SEQ ID NO:48, Q9QZ22, Olfactory receptor- *Mus musculus*
6. SEQ ID NO:49, Q9QZ19, Olfactory receptor- *Mus musculus*

		10	20	30	40	50																																												
GPCR5	M	K	W	A	N	O	T	A	-	V	T	E	V	L	M	G	L	H	E	H	C	N	L	E	V	V	L	F	V	E	C	L	G	I	S	V	N	V	L	G	N	A	L	L	I	G	L	N	
XX: Q9QZ17	M	A	G	T	N	H	T	E	-	V	I	E	V	L	L	G	L	O	D	H	H	G	L	E	I	A	L	F	V	L	C	L	G	I	C	M	T	L	L	G	N	S	F	L	V	G	L	I	
XX: Q9QZ20	M	D	V	S	N	O	I	T	-	V	T	E	F	V	L	L	G	L	S	A	H	P	K	L	E	K	T	F	F	V	L	L	S	M	Y	L	V	I	L	L	G	N	C	V	L	I	L	V	S
XX: Q9QZ21	M	E	G	A	N	O	S	T	-	V	A	E	F	V	L	L	G	L	S	D	H	P	K	L	E	K	T	F	F	V	L	L	M	Y	L	V	I	L	L	G	N	C	V	L	I	L	V	S	
XX: Q9QZ22	M	D	R	S	N	E	T	A	P	L	S	G	F	I	L	L	G	L	S	A	H	P	K	L	E	K	T	F	F	V	L	L	M	Y	L	V	I	L	L	G	N	C	V	L	I	L	V	S	
XX: Q9QZ19	M	E	R	S	N	K	T	P	V	S	S	F	I	L	L	G	L	S	A	H	P	K	L	E	K	T	F	F	V	L	L	M	Y	L	V	I	L	L	G	N	C	V	L	I	L	V	S		
		60	70	80	90	100																																												
GPCR5	V	I	H	P	R	L	N	P	M	Y	F	L	S	N	L	S	L	M	D	I	C	G	T	S	S	F	V	P	L	M	L	D	N	F	L	E	T	Q	R	T	I	S	F	P	G	C	A	L	

5	XX: Q9QZ17	VLDTHLHSPMYFFISNLSLIDICGTSSTPMMLNFDVQRTISFSPSCAL
	XX: Q9QZ20	ILDShLHTPMYFFLGnLSFLDICYTTSSVPLVLDGFLTPRKTI SFSGCAV
	XX: Q9QZ21	ILDShLHTPMYFFLGnLSFLDICYTTSSVPLVLDGFLTPRKTI SFSGCAV
	XX: Q9QZ22	ILDShLHTPMYFFLGnLSFLDICYTTSSVPLVLDGFLTPRKTI SFSGCAV
	XX: Q9QZ19	ILDShLHTPMYFFLGnLSFLDICYTTSSVPLVLDGFLTPRKTI SFSGCAV
10	GPCR5110.....120.....130.....140.....150
	XX: Q9QZ17	QMYLTITLALGSTECLLLAVMAYDRYVAICQPLRYPELVNGRYASRWQDK-L
	XX: Q9QZ20	QMFSLFAMGATECVLLGMMAFDRIYVAICNPLRYPVVMNKAAYVPMVSSW
	XX: Q9QZ21	QMFSLFAMGATECVLLGMMAFDRIYVAICNPLRYPVVMNKAAYVPMVSSW
	XX: Q9QZ22	QMFSLFAMGATECVLLGMMAFDRIYVAICNPLRYPVVMNKAAYVPMVSSW
	XX: Q9QZ19	QMFSLFAMGATECVLLGMMAFDRIYVAICNPLRYPVVMNKAAYVPMVSSW
15	GPCR5160.....170.....180.....190.....200
	XX: Q9QZ17	GTGFANSLQSTILVWHLPFCGHVIN--YFYEILAVLKLACGDISLNALAL
	XX: Q9QZ20	GTGFANSLVQISLAVQLPFCGDNVINHFICEILAVLKLACADISINVISM
	XX: Q9QZ21	GTGFANSLVQISLAVQLPFCGDNVINHFICEILAVLKLACADISINVISM
	XX: Q9QZ22	GTGFANSLVQISLAVQLPFCGDNVINHFICEILAVLKLACADISINVISM
	XX: Q9QZ19	GTGFANSLVQISLAVQLPFCGDNVINHFICEILAVLKLACADISINVISM
20	GPCR5210.....220.....230.....240.....250
	XX: Q9QZ17	TVATAVLTMTPLLLCLSYIFILAAILRVPSAAGRSKAFSTCSAHLTVVV
	XX: Q9QZ20	GVANVIFLGVVPLFIFVSYIFILSTILRIPSAEGRKKAFSTCSAHLTVVI
	XX: Q9QZ21	GVANVIFLGVVPLFIFVSYIFILSTILRIPSAEGRKKAFSTCSAHLTVVI
	XX: Q9QZ22	GVANVIFLGVVPLFIFVSYIFILSTILRIPSAEGRKKAFSTCSAHLTVVI
	XX: Q9QZ19	GVANVIFLGVVPLFIFVSYIFILSTILRIPSAEGRKKAFSTCSAHLTVVI
25	GPCR5260.....270.....280.....290.....300
	XX: Q9QZ17	VFYGTILFMYGKPKSKDPLGADKQDLADKLISLFYGVVTPMLNPIIYSLR
	XX: Q9QZ20	VFYGTILFMYGKPKSKDPLGADKQDLADKLISLFYGVVTPMLNPIIYSLR
	XX: Q9QZ21	VFYGTILFMYGKPKSKDPLGADKQDLADKLISLFYGVVTPMLNPIIYSLR
	XX: Q9QZ22	VFYGTILFMYGKPKSKDPLGADKQDLADKLISLFYGVVTPMLNPIIYSLR
	XX: Q9QZ19	VFYGTILFMYGKPKSKDPLGADKQDLADKLISLFYGVVTPMLNPIIYSLR
30	GPCR5310.....320.....330
	XX: Q9QZ17	NSEVKAAVTALLWGGLLTRKMSHF-----
	XX: Q9QZ20	NKDVKAAVRNLVGQKHTE-----
	XX: Q9QZ21	NKDVKAAVRNLVGQKHTE-----
	XX: Q9QZ22	NKDVKAAVRNLVGQKHTE-----
	XX: Q9QZ19	NKDVKAAVRNLVGQKHTE-----
35	GPCR5340.....350.....360.....370.....380
	XX: Q9QZ17	NKDVKAAVRNLVGQKHTE-----
	XX: Q9QZ20	NKDVKAAVRNLVGQKHTE-----
	XX: Q9QZ21	NKDVKAAVRNLVGQKHTE-----
	XX: Q9QZ22	NKDVKAAVRNLVGQKHTE-----
	XX: Q9QZ19	NKDVKAAVRNLVGQKHTE-----

Table 5F lists the domain description from DOMAIN analysis results against GPCR5. This protein contains domain IPR000276 at amino acid positions 41 to 287. This indicates that the GPCR5 sequence has properties similar to those of other proteins known to contain this domain as well as to the 254 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 5F Domain Analysis of GPCR5

PSSMs producing significant alignments:

Score E
(bits) value

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5 7tm_1      *->GNLLVILVILRTKKLRTPTNIFILNLAVADLLFLLTLPPWALYYLVG
   ||+| | + +++ +|++|+++++ ||++ |++ + + | +| +++
GPCR5 41     GNALLIGLNVLHPRHLNPMYFLLSNLSLMDICGTSSFVPLMLDNFLE 87

   GSEDWPFGSALCKLVTALDVVNMYASILLTALSIDRYLAIVHPLRYRRR
   + ++ +| |+++| ++ + + +| |+++++| |++|+ | | | + +
10 GPCR5 88 --TQRTISFPGCALQMYLTLALGSTECLLAVMAYDRYVAICQPLRYPEL 135

   RTSPRRAKVVILLVWVLALLSLPPLLFSWVKTVEEGNGTLN.....VN
   ++ + ++ + +| | +++ | |++ ++ ++++++|++ + + |
GPCR5 136 MS-GQTCMQMAALSWGTFANSLQSL-VWHLPFCEGHVINYFYELAVL 183

15   VTVCLIDFPEESTASVSTWLRSYVLLSTLVGFLLPLLVLVCYTRILRTL
   + +|+ + + + + + + + + + + + + + + + + + + + + +
GPCR5 184 KLACGDISLNAL-----ALMVATAVLTAPLLLICLSYLFILSAI 223

20   R.....KAAKTLVVVVVFVLCWLPHYFIVLLLDTLCLSIIMSSTCE
   + ++ ++ | + ++ + ++| + ++ + + + + + + + + + +
GPCR5 224 LRVPSAAGRCKAFSTCSAHRTVVVVFYGTISFMYFKPKAKDP----- 265

   LERVLPTALLVTLWLAYVNSCLNPIIY<-*
25   + + | +|++++| + | | | | |
GPCR5 266 -----NVDKTVAFYGVVTPSLNPIIY 287

```

A GPCR-like protein of the invention, referred to herein as GPCR5, is an Olfactory Receptor ("OR")-like protein. Some members of the Olfactory Receptor-Like Protein Family end up localized at the cell surface, where they exhibit activity. Therefore it is likely that these novel GPCR5 proteins are available at the appropriate sub-cellular localization and hence accessible for the therapeutic uses described in this application.

The GPCR disclosed in this invention is expressed in at least in some of the following tissues: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain - hippocampus, brain - substantia nigra, brain - thalamus, brain -whole, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. This information was derived by determining the tissue sources of the sequences that were included in the invention. In addition, the sequence is predicted to be expressed in the following tissues because of the expression pattern of (GENBANK-ID: gb:GENBANK-ID:MMU133430|acc: AJ133430) a closely related or6 gene homolog in species *Mus musculus*: olfactory epithelium. Expression data for GPCR5 is provided in Example 2.

The nucleic acids and proteins of GPCR5 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further above.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the “Anti-GPCR5 Antibodies” section below. The disclosed GPCR5 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR5 epitope is from about amino acids 1 to 25. In another embodiment, a GPCR5 epitope is from about amino acids 120 to 140. In further specific embodiments, GPCR5 epitopes are from about amino acids 230 to 245 and from about amino acids 252 to 280.

GPCR6

A sixth GPCR-like protein of the invention, referred to herein as GPCR6, is an Olfactory Receptor (“OR”)-like protein. Some members of the Olfactory Receptor-Like Protein Family end up localized at the cell surface, where they exhibit activity. Therefore it is likely that these novel GPCR6 proteins are available at the appropriate sub-cellular localization and hence accessible for the therapeutic uses described in this application.

The disclosed novel GPCR6 (alternatively referred to herein as CG 50177-01) includes the 766 nucleotide sequence (SEQ ID NO:50) shown in Table 6A. A GPCR6 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 2-4 and ends with a TAG codon at nucleotides 761-763. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 6A, and the start and stop codons are in bold letters.

Table 6A. GPCR6 Nucleotide Sequence (SEQ ID NO:50)

<u>GTCAGCCTCCAATATCACCTTAACACATCCA</u> ACTGCCTTCTTGGTGGGGATTCCAGGCCTGGAA CACCTGCACATCTGGATCTCCATCCCTTTCTGCTTAGCATGTACACTGGCCCTGCTTGGAAACTGCA CTCTCCTTCTCATCATCCAGGCTGATGCAGCCCTCCATGAACCCATGTACCTCTTTCTGGCCATGTT
--

GGCAGCCATCGACCTGGTCTTTCCTCCTCAGCACTGCCCAAGATGCTTGCCATATTCTGGTTCAGG
GATCGGGAGATAAACTTCTTTGCCTGTCTGGCCAGATGTTCTTCCTTCACTCCTTCTCCATCATGG
AGTCAGCAGTGCTGCTGGCCATGGCCTTTGACCGCTATGTGGCTATCTGCAAGCCACTGCACTACAC
CAAGTCTGACTGGGTCCCTCATCACCAAGATTTTATTGTGGTGTGGACCTGCTCCTTGTATC
CTGTCTTATATCTTTATCTTCAGGCAGTCTACTGCTTGCTCTCAGGAGGCCCGCTACAAGGCAT
TTGGGACATGTGTCTCTCATATAGGTGCCATCTTAGCCTTCTACACAACTGTGGTCATCTCTCAGT
CATGCACCGTGTAGCCCGCCATGCTGCCCCCATGTCCACATCCTCCTTACCAATTTCTATCTGCTC
TTCCCAATGGTCAATCCCATATCTATGGTGTCAAGACCAAGCAAATCCGTGAGAGCATCTTGG
GAGTATTCCCAAGAAAGGATATGTAGAGG

The GPCR6 protein (SEQ ID NO:51 encoded by SEQ ID NO50 is 253 aa in length, has a molecular weight of 28233.48 Daltons, and is presented using the one-letter amino acid code in Table 6B. The Psort profile for GPCR6 predicts that these sequences have a signal peptide and are likely to be localized at the plasma membrane with a certainty of 0.640. The Signal P predicts a likely cleavage site for a GPCR6 peptide is between positions 54 and 55, *i.e.*, at the dash in the sequence ADA-AL.

Table 6B. Encoded GPCR6 protein sequence (SEQ ID NO:51)

SASNITLTHPTAFLLVGIPGLEHLHIWISIPFCLACTLALLGNCTLLLI IQADAALHEPMYLFLAMLA
AIDLVLSSSALPKMLAIFWFRDREINFFACLAQMFFLHSFSIMESAVLLAMAFDRYVAICKPLHYTKV
LTGSLITKIFIVVLDLLLVLSYIFILQAVLLASQEBARYKAFGTCSHIGAILAFYTTVVIVSSVMHR
VARHAAPHVHILLTNFYLLFPMPVNPPIYGVKTKQIRESILGVFPRKDM

Additional SNP variants are disclosed in Example 3.

The amino acid sequence of GPCR6 had high homology to other proteins as shown in Table 6C.

Table 6C. BLASTX results for GPCR6

Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum	
		Prob	P (N)
patp:AAG72254 Human olfactory receptor polypeptide, 313 a.a.	492	2.7e-75	
patp:AAG71568 Human olfactory receptor polypeptide, 319 a.a.	473	5.5e-73	
patp:AAG71839 Human olfactory receptor polypeptide, 314 a.a.	733	2.1e-72	
patp:AAB85003 rat olefactory recep. 320 a.a.	437	2.4e-70	
patp:AAG71643 Human olfactory receptor polypeptide, 313 a.a.	456	2.7e-69	

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of this invention has 278 of 406 bases (68%) identical to a gb:GENBANK-ID:AF121975|acc:AF121975.1 mRNA from Mus musculus (Mus musculus odorant receptor S18 gene, complete cds). The full amino acid sequence of the protein of the invention was found to have 87 of 163 amino acid residues (53%) identical to, and 116 of 163 amino acid

residues (71%) similar to, the 321 amino acid residue ptrn:SPTREMBL-ACC:Q9WU89 protein from Mus musculus (Mouse) (ODORANT RECEPTOR S18).

GPCR6 also has homology to the proteins shown in the BLASTP data in Table 6D.

Table 6D. GPCR6 BLASTP results

Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Q9WU89	Odorant receptor S18/mouse	321	87/163 (53%)	116/163 (71%)	3.5e-70
O88628	Olfactory receptor 51E2/rat	320	82/148 (55%)	105/148 (70%)	3.5e-70
Q9H255	Olfactory receptor 51E2/human	320	80/148 (54%)	106/148 (71%)	7.3e-68
Q9WVD9	MOR 3'BETA1/mouse	326	86/168 (51%)	116/168 (69%)	31.e-67
Q9H346	Olfactory receptor 52D1/human	318	87/166 (52%)	115/166 (69%)	4.0e-67

A multiple sequence alignment is given in Table 6E, with the GPCR6 protein being shown on line 1 in Table 6E in a ClustalW analysis, and comparing the GPCR6 protein with the related protein sequences shown in Table 6D. This BLASTP data is displayed graphically in the ClustalW in Table 6E.

Table 6E. ClustalW Analysis of GPCR6

1. SEQ ID NO:51, GPCR6
2. SEQ ID NO:52, Q9WU89, Odorant receptor S18/mouse
3. SEQ ID NO:53, O88628, Olfactory receptor 51E2/rat
4. SEQ ID NO:54, Q9WVD9, MOR 3'BETA1/mouse 51E2/human
5. SEQ ID NO:55, Q9H255, Olfactory receptor
6. SEQ ID NO:56, Q9H346, Olfactory receptor 52D1/human

```

GPCR6  ---SASNIILTH---PTAFELVIGIPGLEHLHWTISIPFCLACTLALIGN 43
Q9WU89  MNSKASMLGTNFTIIHPTVFLLGIPGLEQYHTWLSIPFCLMYTAAVLGN 50
O88628  --MSSCNFIHAT-----FVLIGIPGLEEAHFWFGFPLLSMYAVALEGN 41
Q9WVD9  MKVASSFHNDTN--PQDVWVVLIGIPGLEDLHSWFAIPTCSMYIVAVLGN 48
Q9H255  --MSSCNFIHAT-----FVLIGIPGLEKAHFWVGFPPLLSMYIVAVLGN 41
Q9H346  --MSDSNLSDNH--LPDT--FVLIGIPGLEAAHFWEAIPFCAMYLEVALVGN 45

GPCR6  CTLLLTQADAALHEPMYLFLAMLAAIDLVLSSALPKMLAIFWFERDREI 93
Q9WU89  GALLIVLSERLHEPMYFLSMLAGTDLLSTTTPKTLAIFWFHAGEI 100
O88628  CIVVFLVRLERSLHAPMYLFLCMLAIDLALSTSTMPKTLAIFWEDSREI 91
Q9WVD9  VLLIFLIVRLERSLHEPMYFLSMLALADLLSTATAPKMLAIFWFHSRGI 98
Q9H255  CIVVFLVRLERSLHAPMYLFLCMLAIDLALSTSTMPKTLAIFWEDSREI 91
Q9H346  AAILLVIAMDNALHAPMYLFLCLLSLTDLALSTTTPKMLAILLHAGEI 95

GPCR6  NFFACLAQMFFIHFSFIMESAVLLAMAFDRYVAICKPLRYKVLTCGLIT 143
Q9WU89  PFDACLAQMFFIHFAFVAESCILLAMAFDRYVAICTPLRYSAVLTPMAIG 150
O88628  PFDACLAQMFFIHSAIESITILLAMAFDRYVAICKPLRYAAVLNNIVTV 141
Q9WVD9  SFGSCVSMQMFHIFHFAESAILLAMAFDRYVAICKPLRYITLTSQVIG 148

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Q9H255 SFEACLTOMFFFIHSAESTILLAMAFDRYVAICPLRHAAVLNNTVTA 141
Q9H346 SFGGCLAOMFCFHSIYALESSILLAMAFDRYVAICNPLRYTTLNHAFIG 145

5 GPCR6 KI-----F 146
Q9WU89 KMTLAIWGRSIGTIFPIIFLKKRLSYGRTNVIPHSYCEHIGVARLACADI 200
O88628 CTGMVALVRGSLFFPLPLLRRLAFCHSNVLSHSYCVHODVMKILAYTDT 191
Q9WVD9 KIGTAAVRSFLICFPFIFLVYRLLYCGKHTPHSYCEHMGTLARLACDNI 198
Q9H255 CIGIVAIVRGSLFFELPLLRRLAFCHSNVLSHSYCVHODVMKILAYADT 191
Q9H346 RICFVGLFHSVAIVSEFIFLKRRLPCHGRVMTHTYCEHMGTLARLACANI 195

10 GPCR6 IVVH-----DLLLVII SYIFILCAVLLLASQBARVKAFGTCV 183
Q9WU89 TVNTIYWGFSVPMASVLVDVALIGISYTHILOAVFRLPSQDAREKALNTICG 250
O88628 LPNVVYGLTALLVMGVDVMTSLSYFLLIRAVLQLPSKSERAKAFGTCV 241
Q9WVD9 TVNTIYGLTALLSTGLDILLLIISYTHILRTVFCIPSWAARYKALNTICG 248
15 Q9H255 LPNVVYGLTALLVMGVDVMTSLSYFLLIRTVLQLPSKSERAKAFGTCV 241
Q9H346 TVNTIYGLTALLVMGVDVMTSLIATSYGFIILHAFVHLP SHDAQH KALSTCG 245

20 GPCR6 SHIGAILAFYTTVVVISVVMHRVARHAAP-HVHILMTNFYLLFPPMVNPIL 232
Q9WU89 SHIGVILLFPIPSFFTFITHRFG-KNIPHHVHILLANLYVLPVPPMLNPIL 299
O88628 SHIGVLLAFYVPLIGLSVHRFGNSLDP-IVHVLMGDVYLLFPPVINPIL 290
Q9WVD9 SHICVILLFYTPAFFFFFAHRFGGKTVPRHSHILANLYVAVPPMLNPIL 298
Q9H255 SHIGVLLAFYVPLIGLSVHRFGNSLHP-IVRVVMGDYLLFPPVNPIL 290
Q9H346 SHIGVILLFYTPAFFFFTHRFCHHEVPRHSHILANLYVLPVPPMLNPIL 295

25 GPCR6 YGVKTKQIRESILGVFPRKDM----- 253
Q9WU89 YGAKTKQIRDSMTIRMLSVVWKS----- 321
O88628 YGAKTKQIRTRMLAMEKISCDKDIEAGGNT 320
Q9WVD9 YGVKTKQIDRVVFLFSSVSTCQHD SRC-- 326
Q9H255 YGAKTKQIRTRMLAMEKISCDKDLQAVGGK 320
30 Q9H346 YGAKTKQIRSRMLKLLHLGKTSI----- 318

Table 6F lists the domain description from DOMAIN analysis results against GPCR6. This indicates that the GPCR6 sequence has properties similar to those of other proteins known to contain this domain as well as to the 254 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 6F Domain Analysis of GPCR6

PSSMs producing significant alignments:

Score E
(bits) value
53.3 1e-08

gnl|Pfam|pfam00001 7tm_1, 7 transmembrane receptor (rhodopsin family)

GPCR6: 56 LHEPMYLFMLAMLAIDLVLSSALPKMLAIFWFRDREINFFACLAQMFFLHSFSIMESAV 115
7tm_1: 15 LRTPTNIFLLNLAVADLLFLLTLPPWALYYLVGGDWVFGDALCKLVGALFVVNGYASILL 74

GPCR6: 116 LLAMAFDRYVAICKPLHYTKVLT 138 (SEQ ID NO.51)
7tm_1: 75 LTAISIDRYLAIVHPLRYRRIRT 97 (SEQ ID NO: 18)

The GPCR6 disclosed in this invention is expressed in at least the following tissues:

Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver,

fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR6 is provided in Example 2.

The nucleic acids and proteins of GPCR6 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further above.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR_X Antibodies" section below. The disclosed GPCR6 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR6 epitope is from about amino acids 2 to 50. In another embodiment, a GPCR6 epitope is from about amino acids 50 to 80. In further specific embodiments, GPCR6 epitopes are from about amino acids 90 to 100, from about amino acids 100 to 175, from about amino acids 180 to 210 and from about amino acids 220 to 230.

GPCR7

A further GPCR-like protein of the invention, referred to herein as GPCR7, is an Olfactory Receptor ("OR")-like protein. The novel GPCR7 nucleic acid sequences were identified on chromosome 9 as described in Example 1. Some members of the Olfactory Receptor-Like Protein Family end up localized at the cell surface, where they exhibit activity. Therefore it is likely that these novel GPCR7 proteins are available at the appropriate sub-cellular localization and hence accessible for the therapeutic uses described in this application.

Two alternative novel GPCR7 nucleic acids and encoded polypeptides are provided, namely GPCR7a and GPCR7b.

GPCR7a

In one embodiment, a GPCR7 variant is the novel GPCR7a (alternatively referred to herein as CG50201-01), which includes the 1000 nucleotide sequence (SEQ ID NO:57) shown in Table 7A. A GPCR7a ORF begins with a Kozak consensus ATG initiation codon at nucleotides 3-5 and ends with a TGA codon at nucleotides 951-953. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 7A, and the start and stop codons are in bold letters.

Table 7A. GPCR7a Nucleotide Sequence (SEQ ID NO:57-)

CCATGGAGGCTGCCAATGAGTCTTCAGAGGGAATCTCATTCGTTTTATTGGGACTGACAACAAGTCC
TGGACAGCAGCGGCTCTCTTTGTGCTGTTCTTGCTCTTGATGTGGCCAGCCTCCTGGGCAATGGA
CTCATTGTGGCTGCCATCCAGGCCAGTCCAGCCCTTCATGCACCCATGTAATTCCTGCTGGCCCACC
TGTCCTTTGCTGACCTCTGCTTCGCCTCCGTCACTGTGCCCAAGATGTTGGCCAACCTGTTGGCCCA
TGACCACTCCATCTCGCTGGCTGGCTGCCTGACCCAAATGTACTTCTTCTTTGCCCTGGGGGTAAC
GATAGCTGTCTTCTGGCGGCCATGGCCTATGACTGCTACGTGGCCATCCGGCACCCCTCCCCTATG
CCACGAGGATGTCCCGGGCCATGTGCGCAGCCCTGGTGGGAATGGCATGGCTGGTGTCCCACGTCCA
CTCCCTCCTGTATATCCTGCTCATGGCTCGCTTGTCTTCTGTGCTTCCCACCAAGTGCCCCACTTC
TTCTGTGACCACCAGCCTCTCTTAAGGCTCTCGTGCTCTGACACCCACCACATCCAGCTGCTCATCT
TCACCGAGGGCGCCGAGTGGTGGTCACTCCCTTCTGCTCATCCTCGCCTCCTATGGGGCCATCGC
AGCTGCCGTGCTCCAGCTGCCCCTCAGCCTCTGGGAGGCTCCGGGCTGTGTCCACCTGTGGCTCCCAC
CTGGCTGTGGTGAGCCTCTTCTATGGGACAGTCATTGCAGTCTACTTCCAGGCCACATCCCGACGCG
AGGCAGAGTGGGGCCGTGTGGCCACTGTCATGTACACTGTAGTCACCCCCATGCTGAACCCCATCAT
CTACAGCCTCTGGAATCGCGATGTACAGGGGGCACTCCGAGCCCTTCTCATTGGGCGAAGGATCTCA
GCTAGTGACTCTGAGGGCAGGACCCCACTGAGGACAGACTGCATCACCCACACTGGCAACT

The GPCR7 protein (SEQ ID NO:58) encoded by SEQ ID NO:57 has 316 amino acid residues and is presented using the one-letter code in Table 7B. The predicted molecular weight of GPCR7 protein is approximately 34266.42 Daltons. The Psort profile for GPCR7 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.4600. The Signal P predicts a likely cleavage site between positions 42 and 43, *i.e.*, at the dash in the sequence LLG-NG.

The DNA sequence and protein sequence of GPCR7a was obtained by exon linking as described in the Example 1.

Table 7B. Encoded GPCR7a protein sequence (SEQ ID NO:58)

MEAA NESSEGISFVLLGLTTS PGQQRPLFVLFLLLYVASLLGNGLIVAAIQASPALHAPMYFLLAHL SFA
DLCFASVTVPKMLANLLAHDHSISLAGCLTQMYFFFALGVTDSCLLAAMAYDCYVAIRHPLPYATRMSRA
MCAALVGMAWLVSHVHSLLYILLMARLSFCASHQVPHFFCDHQP LLRLSCSDTHHIQLLIIFTEGA AVVVT
PFLILILASYGAIAAAVLQLPSASGR LRAVSTCGSHLAVVSLFYGT VIAVYFQATSRR EAEWGRVATVMYT
VVTPLNPIIYSLWNRDVQ GALRALLIGRRISASDS

Additional SNP variants are disclosed in Example 3.

GPCR7b

In an alternative embodiment, a GPCR7 variant is the novel GPCR7b (alternatively referred to herein as CG50257-01 or CG40267-01), which includes the 991 nucleotide sequence (SEQ ID NO:59) shown in Table 7C. The GPCR7b ORF begins with a Kozak consensus ATG initiation codon at nucleotides 3-5 and ends with a TGA codon at nucleotides 951-953, which are in bold letters in Table 4C.

Table 7C. GPCR7b Nucleotide Sequence (SEQ ID NO:59)

CCATGGAGGCTGCCAATGAGTCTTCAGAGGGAATCTCATTCGTTTTATGGGACTGACAACAAGTCC
TGGACAGCAGCGGCCTCTCTTTGTGCTGTTCTTGCTCTTGTATGTGGCCAGCCTCCTGGGTAATGGA
CTCATTGTGGCTGCCATCCAGGCCAGTCCAGCCCTTCATGCACCCATGTACTTCCTGCTGGCCACC
TGTCCTTTGCTGACCTCTGCTTCGCCTCCGTCACTGTGCCAAAGATGTTGGCCAACTTGTGGCCCA
TGACCACTCCATCTCGCTGGCTGGCTGCCTGACCCAAATGTACTTCTTCTTGCCCTGGGGGTA
GATAGCTGTCTTCTGGCGGCCATGGCCTATGACTGCTACGTGGCCATCCGGCACCCTCCCTATG
CCACGAGGATGTCCCGGGCCATGTGCGCAGCCCTGGTGGGAATGGCATGGCTGGTGTCCACGTCCA
CTCCCTCCTGTATATCCTGCTCATGGCTCGCTTGCTCTCTGTGCTTCCACCAAGTGCCCACTTC
TTCTGTGACCACAGCCTCTCTTAAGGCTCTCGTGCTCTGACACCCACCACATCCAGCTGCTCATCT
TCACCGAGGGCGCCGAGTGGTGGTCACTCCCTTCTGCTCATCCTCGCCTCCTATGGGGCCATCGC
AGCTGCCGTGCTCCAGCTGCCCTCAGCCTCTGGGAGGCTCCGGGCTGTGTCCACCTGTGGCTCCAC
CTGGCTGTGGTGAGCCTCTTCTATGGGACAGTCATTGCAGTCTACTTCCAGGCCACATCCGACGCG
AGGCAGAGTGGGGCCGTGTGGCACTGTCTGTACACTGTAGTCAACCCCATGCTGAACCCCATCAT
CTACAGCCTCTGGAATCGCGATGTACAGGGGGCACTCCGAGCCCTTCTCATTTGGGCGAAGGATCTCA
GCTAGTGACTCCT**GAGGGCAGGACCCCACTGAGGACAGACTGCATCACCACA**

The GPCR7b protein (SEQ ID NO:60) encoded by SEQ ID NO:59 is 316 amino acids in length, has a molecular weight of 34266.42 Daltons, and is presented using the one-letter code in Table 7D. As with GPCR7a, the most likely cleavage site for a GPCR7b peptide is between amino acids 42 and 43, i.e., at the dash in the sequence LLG-NG, based on the SignalP result. The DNA sequence and protein sequence of GPCR7a was obtained by exon linking as described in the Example 1.

Table 7D. GPCR7b protein sequence (SEQ ID NO:60)

MEANESSEGISFVLLGLTTSPGQQRPLFVLFLLLYVASLLGNGLIVAAIQASPALHAPMYFLLAHL
SFADLCFASVTVPKMLANLLAHDHSISLAGCLTQMYFFFALGVTDSCLLAAMAYDCYVAIRHPLPYA
TRMSRAMCAALVGMAWLVSHVHSLLYILLMARLSFCASHQVPHFFCDHQPLLRLSCSDTHIQLLIF
TEGAAVVVPFLLILASYGAIAAVALQLPSASGRRAVSTCGSHLAVVSLFYGTIVAVYFQATSRRE
AEWGRVATVMYTVVTPMLNPITYSLWNRDVQALRALLIGRRISASDS

GPCR7 Clones

The amino acid sequence of GPCR7 had high homology to other proteins as shown in Table 7e.

Table 7e. BLASTX results for GPCR7

Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Prob P (N)
ptnr:TREMBLNEW-ACC:AAK95107 OLFACTORY RECEPTOR - Homo sap...	1115	1.0e-112
ptnr:SWISSPROT-ACC:P23266 OLFACTORY RECEPTOR-LIKE PROTEIN...	870	9.3e-87
ptnr:pir-id:B23701 olfactory receptor F5 - rat, 313 aa.	864	4.0e-86
ptnr:SWISSNEW-ACC:O43749 Olfactory receptor 1F1 (Olfactor...	859	1.4e-85
ptnr:SPTREMBL-ACC:Q9TUA1 OLFACTORY RECEPTOR - Pan troglod...	801	1.9e-79

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of this invention has 592 of 880 bases (67%) identical to a gb:GENBANK-ID:RATOLFPROC|acc:M64377.1 mRNA from Rattus norvegicus (Rat olfactory protein mRNA, complete cds). The full amino acid sequence of the GPCR7 was found to have 174 of 309 amino acid residues (56%) identical to, and 223 of 309 amino acid residues (72%) similar to, the 313 amino acid residue ptnr:pir-id:B23701 protein from rat (olfactory receptor F5).

GPCR7 also has homology to the proteins shown in the BLASTP data in Table 7F.

Table 7F. GPCR7 BLASTP results

Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
AAK95107	Olfactory receptor/human	216	216/216 (100%)	216/216 (100%)	1e-112
P23266	Olfactory-like protein F5/rat	313	175/309 (56%)	224/309 (72%)	9.3e-87
B23701	Olfactory-like protein F5/rat	313	174/309 (56%)	223/309 (72%)	4.0e-86
AC 043749	Olfactory receptor 1F1/human	312	171/309 (55%)	219/309 (70%)	1.4e-85

Q9TUA1	Olfactory receptor/chimpanzee	315	159/310 (51%)	211/310 (76%)	1.9e-79
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A multiple sequence alignment is given in Table 7G, with the GPCR7 protein being shown on line 1 in Table 7G in a ClustalW analysis, and comparing the GPCR7 protein with the related protein sequences shown in Table 7F. This BLASTP data is displayed graphically in the ClustalW in Table 7G. The amino acid residues of GPCR7a and GPCR7b are identical.

Table 7G. ClustalW Analysis of GPCR7

1.	SEQ ID NO:58,	GPCR7a	
2.	SEQ ID NO:60,	GPCR7b	
3.	SEQ ID NO:61,	AAK95107	
4.	SEQ ID NO:62,	P23266	
5.	SEQ ID NO:63,	B23701	
6.	SEQ ID NO:64,	AC 043749	
7.	SEQ ID NO:65,	Q9TUA1	
15	GPCR7a	MEAA NESSEGISFVLLGLITSEGGQORPLFVLFLLLYVASLLGNGLI VAAI	50
	GPCR7b	MEAA NESSEGISFVLLGLITSEGGQORPLFVLFLLLYVASLLGNGLI VAAI	50
	AAK95107	-----	1
	P23266	MSSTN OSS-VTEFILLGLSRQPQQQLLFLFLIMYLATVLGNLLIILAI	49
	B23701	MSSTN OSS-VTEFILLGLSRQPQQQLLFLFLIMYLATVLGNLLIILAI	49
20	AC 043749	MSGTN OSS-VSEFILLGLSRQPQQQLLFLFLIMYLATVLGNLLIILSV	49
	Q9TUA1	MMGQN OSS-ISDFILLGLPIQPEQQNLCYALFLAMYLITLLGNLLIITVLI	49
25	GPCR7a	QASPALHAPMYFLLAHLSEADICFASVTVPKMLANLLAHDHSISLAGCLT	100
	GPCR7b	QASPALHAPMYFLLAHLSEADICFASVTVPKMLANLLAHDHSISLAGCLT	100
	AAK95107	-----FADICFASVTVPKMLANLLAHDHSISLAGCLT	32
	P23266	GTDSRLHITPMYFFLSNLSFVDVCFSSSTTVPKVLNHLGSAISFSGCLT	99
	B23701	GTDSRLHITPMYFFLSNLSFVDVCFSSSTTVPKVLNHLGSAISFSGCLT	99
	AC 043749	SIDSRLHITPMYFFLSNLSFVDVCFSSSTTVPKVLNHLLETQTISFCGCLT	99
	Q9TUA1	RLDSRLHITPMYFFLSNLSFSDICFSSSVTTPKLLQNMQNQDPSIPYADCLT	99
30	GPCR7a	QMYFFFALGVIDSCLLAAMAYDCVVAIRHPLPYATRM SRAMCAALVGMAW	150
	GPCR7b	QMYFFFALGVIDSCLLAAMAYDCVVAIRHPLPYATRM SRAMCAALVGMAW	150
	AAK95107	QMYFFFALGVIDSCLLAAMAYDCVVAIRHPLPYATRM SRAMCAALVGMAW	82
	P23266	QLYFLAVFCNMDN FLLAVMSYDRFVAICHPLHYTKMTROL CVLLVVGSW	149
	B23701	QLYFLAVFCNMDN FLLAVMSYDRFVAICHPLHYTKMTROL CVLLVVGSW	149
35	AC 043749	QMYFVFMFVMDN FLLAVMAYDHEVAVCHPLHYTKMTROL CVLLVVGSW	149
	Q9TUA1	QMYFLLFCDLSEFLLVAMAYDRVAICFPLHYTAIMSPMLCLSLVALSW	149
40	GPCR7a	LVSHVHSLLYILLMARLSFCASHQVPHFFCDHQPLRLSCSDTHHIQLI	200
	GPCR7b	LVSHVHSLLYILLMARLSFCASHQVPHFFCDHQPLRLSCSDTHHIQLI	200
	AAK95107	LVSHVHSLLYILLMARLSFCASHQVPHFFCDHQPLRLSCSDTHHIQLI	132
	P23266	VVANMNCLEHILLMARLSFCADNMIPHFFCDGTPLRLSCSDTHLNEELI	199
	B23701	VVANMNCLEHILLMARKSFCADNMIPHFFCDGTPLRLSCSDTHLNEELI	199
	AC 043749	VVANLVLLITLLMAPLSFCADNATTHFFCDVTPLRLSCSDTHLNEELI	199
45	Q9TUA1	VLITTFHALLITLLMARLCFCADNVIPHFFCDMSALLKLACSDTRVNEWVI	199
50	GPCR7a	FTEGAAVVTPFLLILASYGATAAAVLQLPASGRRAVSTCGSHLAVVS	250
	GPCR7b	FTEGAAVVTPFLLILASYGATAAAVLQLPASGRRAVSTCGSHLAVVS	250
	AAK95107	FTEGAAVVTPFLLILASYGATAAAVLQLPASGRRAVSTCGSHLAVVS	182
	P23266	LTEGAAVVTPFVCILISYIHITCAVLRVSSPRGGWKSFSSTCGSHLAVVC	249
	B23701	LTEGAAVVTPFVCILISYIHITCAVLRVSSPRGGWKSFSSTCGSHLAVVC	249

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AC 043749  LSEGALVYITPFLCILASYMHITCTVLKVPSTKGRWKAFTSTCGSHLAVVL 249
Q9TUA1      FIMCGLLIVVIPPFLLLILGSYARIVSSILKVPSSKGICKAFSTCGSHLSVVS 249

GPCR7a     LFYGTVIIVYFQATSRREAEWGRVATVMYTVVTPMLNPFIYSLWNRDVOG 300
GPCR7b     LFYGTVIIVYFQATSRREAEWGRVATVMYTVVTPMLNPFIYSLWNRDVOG 300
AAK95107   LFYGTVIIVYFQATSRREAEWGRVATVMYTVVTP----- 216
P23266     LFYGTVIIVYFNPSSSHLAGRDMAAAVMYAVVTPMLNPFIYSLRNSDMKA 299
B23701     LFYGTVIIVYFNPSSSHLAGRDMAAAVMYAVVTPMLNPFIYSLRNSDMKA 299
AC 043749  LFYSTIIIVYFNPSSSHSAEKDTMATVLYTVVTPMLNPFIYSLRNRYLKG 299
Q9TUA1     LFYGTIIICILYLCPSANSSTLKETVMAMMYTVVTPMLNPFIYSLRNRMKG 299

GPCR7a     ALRALIIGRRISASDS 316
GPCR7b     ALRALIIGRRISASDS 316
AAK95107   ----- 216
P23266     ALRKVLAMRFPSKQ-- 313
B23701     ALRKVLAMRFPSKQ-- 313
AC 043749  ALKKVVGRRVFSV--- 312
Q9TUA1     ALERVIXKRKNPFL- 314

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Table 7H lists the domain description from DOMAIN analysis results against GPCR7.

This indicates that the GPCR7 sequence has properties similar to those of other proteins known to contain this domain as well as to the 254 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 7H Domain Analysis of GPCR7

PSSMs producing significant alignments:				Score	E
				(bits)	value
gnl Pfam pfam00001	7tm_1, 7 transmembrane receptor (rhodopsin family)			105	5e-24
GPCR7:	42	GNGLIVAAIQASPALHAPMYFLLAHLFSADLCFASVTVPKMLANLLAHDHSISLAGCLTQ		101	
7tm_1:	1	GNLLVILVILRTKKLRTPTNIFLLNLAVADLLFLLTLPPWALYYLVGGDWVFGDALCKLV		60	
GPCR7:	102	MYFFFALGVTDSCLLAAMAYDCYVAIRHPLPYATRMSRAMCAALVGMALVSHVHSLLYI		161	
7tm_1:	61	GALFVVNGYASILLTLAISIDRYLAIVHPLRYRRIPTPRRAKVLILLVWVLALLSLPPL		120	
GPCR7:	162	LLMARLSFCASHQVPHFFCDHQPLLRLSCSDTHHIQLLIFTEGAADVTPFLILILASYGA		221	
7tm_1:	121	LFSWLRTVEEGNTTVCLIDFPEESVKR----SYVLLSTLVG-----FVLPLLVLVCYTR		171	
GPCR7:	222	IAAAV-----LQLPSASGRILRAVSTCGSHLAVVSLFYGTVIIVYFQA----TSRRE		268	
7tm_1:	172	ILRTLKRKRARSQRSLKRSSSERKAAKMLLVVVVVFVLCWLPYHIVLLLDLCLLSIWRV		231	
GPCR7:	269	AEWGRVATVMYTVVTPMLNPFIY 291 (SEQ ID NO: 58, 60)			
7tm_1:	232	LPTALLITLWLAYVNSCLNPFIY 254 (SEQ ID NO: 18)			

The GPCR7 protein predicted here is similar to the “Olfactory Receptor-Like Protein Family”, some members of which end up localized at the cell surface where they exhibit activity. Therefore, it is likely that this novel GPCR7 protein is available at the appropriate

sub-cellular localization and hence accessible for the therapeutic uses described in this application.

The Olfactory Receptor-like GPCR7 proteins disclosed is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR7 is provided in Example 2.

This is by no way limiting in that olfactory receptors are a class of G protein-coupled receptor which are known to be expressed in all tissue types. Further tissue expression analysis is provided in the Examples.

The nucleic acids and proteins of GPCR7 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further herein.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. The disclosed GPCR7 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR7 epitope is from about amino acids 10 to 80. In additional embodiments, GPCR7 epitopes are from about amino acids 100 to 125, from about amino acids 130 to 160, from about amino acids 180 to 240 and from about amino acids 270 to 285.

GPCR8

The disclosed GPCR8 (also referred to as CG50193-01) includes the 1022 nucleotide sequence (SEQ ID NO:66) shown in Table 8A. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 34-36 and ending with a TAG codon at nucleotides 1013-1015. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 8A, and the start and stop codons are in bold letters.

Table 8A. GPCR8 Nucleotide Sequence (SEQ ID NO:66)

TCTCTGTTTCCTCAGGGATTGAGAAAGGGGACA**ATG**TGGCAGAAGAATCAGACCTCTCTGGCAGACT
TCATCCTTGAGGGGCTCTTCGATGACTCCCTTACCCACCTTTTCCTTTTCTCCTTGACCATGGTGGT
CTTCCTTATTGCGGTGAGTGGCAACACCCTCACCATTCTCCTCATCTGCATTGATCCCCAACTTCAT
ACACCAATGTATTTCTGCTCAGCCAGCTCTCCCTCATGGATCTGATGCATGTCTCCACAATCATCC
TGAAGATGGCTACCAACTACCTATCTGGCAAGAAATCTATCTCCTTTGTGGGCTGTGCAACCCAGCA
CTTCCTCTATTTGTGTCTAGGTGGTGTGAATGTTTTCTCTTAGCTGTCTATGCTATGACCGCTAT
GTTGCCATCTGTCTCATCCACTGCGCTATGCTGTGCTCATGAACAAGAAGGTGGGACTGATGATGGCTG
TCATGTCATGGTTGGGGGCATCCGTGAACCTCCCTAATTCACATGGCGATCTTGATGCACCTCCCTTT
CTGTGGGCTCGGAAAGTCTACCACTTCTACTGTGAGTTCCCAGCTGTTGTGAAGTTGGTATGTGGC
GACATCACTGTGTATGAGACCACAGTGTACATCAGCAGCATTCTCCTCCTCCTCCCCATCTTCCTGA
TTTCTACATCCTATGTCTTCATCCTTCAAAGTGTCAATCAGATGCGCTCATCTGGGAGCAAGAGAAA
TGCCTTTGCCACTTGTGGCTCCACCTCACGGTGGTTTCTCTTTGGTTTGGTGCCTGCATCTTCTCC
TACATGAGACCCAGGTCCCAGTGCACCTCTATTGCAGAACAAAGTTGGTTCTGTGTTCTACAGCATCA
TTACGCCACATTGAATTCTCTGATTATACTCTCCGGAATAAAGATGTAGCTAAGGCTCTGAGAAG
AGTGTGAGGAGAGATGTTATACCCAGTGCATTCAACGACTGCAATTGTGTTGCCCGAGTGTAG
AGTGGAA**TAG**GATAAGC

The disclosed GPCR8 nucleic acid sequence of this invention has 574 of 908 bases (63%) identical to a *Canis familiaris* olfactory receptor (CfOLF3) gene (gb:GENBANK-ID:CFU53681|acc:U53681.1)(E = 1.3e-45).

The GPCR8 protein (SEQ ID NO:67) encoded by SEQ ID NO:66 is 323 aa in length and is presented using the one-letter amino acid code in Table 8B. The Psort, SignalP and/or Hydropathy results predict that GPCR8 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, a GPCR8 polypeptide is located to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the microbody (peroxisome) with a certainty of 0.3000. The SignalP shows a signal sequence is coded for in the first 45 amino acids with a likely cleavage site at between positions 45 and 46, at TLT-IL.

Table 8B. Encoded GPCR8 protein sequence (SEQ ID NO:67)

MWQKNQTSIADFILEGLFDDSLTHLFLFSLTMVFLIAVSGNTLTILLICIDPQLHTPMYFLLSQLSL

MDLMHVSTIILKMATNYLSGKKSISFVGCATQHFLYLCLGGAECSLLAVMSYDRYVAICHPLRYAVLM
 NKKVGLMMAVMSWLGASVNSLIHMAILMHFPFCGPRKVYHFYCEFPVAVKLVCGDITVYETTVYISSI
 LLLLPFIPLISTSYVFILQSVIQMRSSGSKRNAFATCGSHLTVVSLWFGACIFSVMRPRSQCTLLQNKV
 GSVFYSIITPTLNSLIYTLRNKDVAKALRRVLRRDVITQCIQRLQLWLPRV

The full amino acid sequence of the protein of the invention was found to have 137 of 305 amino acid residues (44%) identical to, and 205 of 305 amino acid residues (67%) similar to, the 313 amino acid residue ptnr:SPTREMBL-ACC:O76000 protein from Homo sapiens (Human) (DJ80I19.1 OLFACTORY RECEPTOR-LIKE PROTEIN (HS6M1-1)).

- 5 Additional SNP variants of GPCR8 are disclosed in Example 3. The amino acid sequence of GPCR3 has high homology to other proteins as shown in Table 8C.

Table 8C. BLASTX results for GPCR8			
		High Score	Smallest Sum Prob P (N)
Sequences producing High-scoring Segment Pairs:			
patp:AAE04578	Human G-protein coupled receptor-34 protein, 323 aa	1662	7.3e-171
patp:AAG72019	Human olfactory receptor polypeptide, 318 aa	1636	4.1e-168
patp:AAG72165	Human olfactory receptor polypeptide, 318 aa	1631	1.4e-167
patp:AAG72348	Human OR-like polypeptide query sequence, 318 aa	1631	1.4e-167

GPCR8 also has homology to the proteins shown in the BLASTP data in Table 8D.

Table 8D. GPCR8 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
O43869	Olfactory receptor 2T1 (Olfactory receptor 1-25) (OR1-25) - <i>Homo sapiens</i>	311	157/305 (51%)	211/305 (69%)	3.1e-79
CAC34662	Sequence 1 from Patent WO0114554 - <i>Homo sapiens</i>	315	149/303 (49%)	203/303 (66%)	7.9e-74
O76000	Olfactory receptor 2B3 (Olfactory receptor 6-4) (OR6-4) (Hs6M1-1) <i>Homo sapiens</i>	313	137/305 (44%)	205/305 (67%)	4.6e-69
Q9EPF6	T3 OLFACTORY RECEPTOR - <i>Mus musculus</i>	315	144/308 (46%)	196/308 (63%)	5.3e-68
Q95156	OLFACTORY RECEPTOR-LIKE PROTEIN OLF3 - <i>Canis familiaris</i>	317	136/301 (45%)	205/301 (68%)	8.7e-68

10

A multiple sequence alignment is given in Table 8E, with the GPCR8 protein being shown on line 1, in a ClustalW analysis comparing GPCR8 with the related protein sequences disclosed in Table 8D.

Table 8E. ClustalW Analysis of GPCR8

1. SEQ ID NO:67, GPCR8
2. SEQ ID NO:68, O43869
3. SEQ ID NO:69, CAC34662
4. SEQ ID NO:70, O76000
5. SEQ ID NO:71, Q9EPF6
6. SEQ ID NO:72, Q95156

10		10	20	30	40	50
	GPCR8	MWQK-NQTS	LADFILEGLFDDSLTHLFLFSLTMVVFLIAVSCNTLTILLI			
	O43869	MEEY-NTS	STD-FTFMGLFNRKETSGLIFAIISIIFFTALMANGVMIFLI			
	CAC34662	MGRWVNQSY	TDGFFLLGIFSHSQTDLVLFSAVMVFTVALCGNVLLIFLI			
15	O76000	MNWE-NESS	PKFEIILLGFSDRAWLQMPLEFVLLISYITITIFCNVSIMMVC			
	Q9EPF6	MEVCNSTLR	SG-FILMGILDNDPFELLCATITALYLLALTSNGLLLLVH			
	Q95156	MGTG-NQT	WVREVFLLGLSSDWDIEVSLFVLFLITYMVTVLGNFLIILLI			
20		60	70	80	90	100
	GPCR8	CIDPOLHTP	MYFLLSQLSLMDLMHVSTIILKMATNYLSGKKSISFVGCAT			
	O43869	QTDRLRLHT	PMYFLLSHLSLIDMMYISTIVPKMLVNYLLDQRTISFVGCTA			
	CAC34662	YLDAGLHTP	MYFLLSQLSLMDLMVLCNIVPKMAANFLSGRKSISFVGC			
	O76000	YLDPKLHTP	MYFLLTNLSILDLCYTTTITVPHMLVNIGCNKKTISYAGCVA			
25	Q9EPF6	TMDTRLHV	PMYLLWQLSLMDLLLTSVITPKAILDYLLKDNTISFGGCAL			
	Q95156	RLDSRLHTP	MYFLLTNLSLVDVSYATSLIPQMLAHLAAHKAIPFVSCAA			
30		110	120	130	140	150
	GPCR8	QHFLYLCLG	GAECFLLAVMSYDRYVAICHPLRYAVLMNKKVGLMMAVMSW			
	O43869	QHFLYLTLV	GAEEFLLGLMAYDRYVAICNPLRYPVLMSSRRVCWMIIAGSW			
	CAC34662	QIGFFVSLV	GSFGLLLGLMAYDHYVAVSHPLHYPIILMNQRVCLQITGSSW			
	O76000	HLIIFLA	LGATECLLLAVMSFDRYVAVCRPLHYVVIIMNYWFCRMAAFSW			
	Q9EPF6	QMFLALTLG	TAE DLLLSFMAYDRYVAICHPLNYTILMSQKVCCLMIATSW			
35	Q95156	QLFFSLGLG	GIEFVLLAVMAYDRYVAVCDPLRYSVIMHGGLCTRLAITSW			
40		160	170	180	190	200
	GPCR8	LGASVNSLI	HMAILMHFPFCGPRKVYHFFCEFPVAVVKLVCGDITVYETTV			
	O43869	FGGSLDG	FLLPITMSFPFCNSREINHFFCEAPAVLKLACADTALYETVM			
	CAC34662	AFGIIDGVI	QMVAAAGLPYCGSRSDHFFCEVQALLKLACADTSLFDTLL			
	O76000	LIGFGNSV	LQSSLTLNMPRCCHQEVDHFFCEVPALLKLSCADTKPIEAEAL			
	Q9EPF6	SLASLSAL	GYSMYTMQYPPFCKSRQIRELFCBIPLLLKLACADTSTYELMV			
	Q95156	VSGSMNSL	MQTVITFQLPMCTNKYIDHISCELLAVVRLACVDTSSNEIAI			
45		210	220	230	240	250
	GPCR8	YISS-IL	LLLPIFLITSYVFI	LQSVIQMRSSGSKRNAFATCGSHLTVVVS		
	O43869	YVCCVLML	LIPFSVVLASYARILTTVQCMSSVEGRKKAFATCSSHMTVVVS			
	CAC34662	FACCVFML	LILPFSITMASYACILGAVLRIRSAQAWKKALATCSSHLTAVT			
	O76000	FFFSVLIL	LIPVTTILISYGFI	AQAVLKIRSAEGRQKAFGTCCSHMIVVS		
	Q9EPF6	YLMGVTL	LFPALAILASYS	LILFTVLHMP	SNEGRRKALVTCSSHLTVVG	
	Q95156	MVSSIVLL	MTFCLVLLSYIQI	ISTILKIQSTEG	RKKAFHTCASHLTVVVS	
55		260	270	280	290	300
	GPCR8	LWFGACIF	SYMRRPSQCTLLQ	NKVGSVFYSLITPTL	NSLIYTLRNKDVAK	

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O43869  LFYGAAMYTYMLPHSYHKPAQDKVLSVFYITLTPMLNPLIYSLRNKDVIG
CAC34662 LFYGAAMFMYLRPRRYRAPSHDKVASIFYTVLTPMLNPLIYSLRNGEVMG
O76000  LFYGTATYMYLQPPSSSTSKDWGKMVSLFYGTITSMNLNPLIYSLRNKDMKE
Q9EPF6  MWYGGALVMYVLPSSFHSPKQDNISSVFYITFTPALNPLIYSLRNKEVIG
5  Q95156  LCYGMALFTYIQPRSSPSVLQEKLLISLFYSVLTPMLNPMIYSVRNKEVKG

                                310      320
                                .....|.....|.....|.....|
10  GPCR8    ALRRVLRRDVITQCIQRLQLWLPRV
    O43869  ALKRALGR-----FK-GPQ-----
    CAC34662 ALRKGLDR-----CRIGSQH-----
    O76000  AFKRLMPR----IFFCKK-----
    Q9EPF6  ALRRVLGKR-----LSVQSTF-----
15  Q95156  AWQKLIGQ----LTGITSKLAT---

```

Table 8F lists the domain description from DOMAIN analysis results against GPCR8. The results indicate that GPCR8 contains the protein domain 7tm_1(InterPro)7 transmembrane receptor (rhodopsin family) at amino acid positions 41 to 289. This indicates that the GPCR8 sequence has properties similar to those of other proteins known to contain this domain as well as to the 254 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 8F Domain Analysis of GPCR8			
PSSMs producing significant alignments:		Score (bits)	E value
gnl Pfam pfam00001	7tm_1, 7 transmembrane receptor (rhodopsin family)	122.3	8.3e-38

```

25  GPCR8    41  GNLLVILVILRTKKLRTPTNIFILNLAVADLLFLLTLPWPALYYLVG
    || | +++++ ++++++++ |++ ||+ + + + ++++++
    GNTLTILLICIDPQLHTPMYFLLSQLSLMDLMHVSTIILKMATNYLS 87

30  GPCR8    88  GSEDWPFGSALCKLVTALDVVNMYASILLTASIDRYLAIVHPLRYRRR
    + + ++ ++| ++++++++ + + +++|+++|+|||+||++|+++ ++
    G--KKSISFVGCATQHFLYLCLGGAECFLLAVMSYDRYVAICHPLRYAVL 135

35  GPCR8    136 RTSPRAKVIVLLVWVLLALLSLPPLLFSWVKTVEEGNGTLNVNVTVCLI
    ++ +++++ + + | + + + +++++ + +++++++ ++ +++++| +
    MN-KKVGLMMAVMSWLGASVNSLIH-MAILMHFPFCGPRK--VYHFYCEF 181

40  GPCR8    182 DFPEESTASVSTWLRSYVLLSTLVGFLLPLLVLVCYTRILRTLRL....
    + +++ + + +++++ + ++|+++++ | +|+ + + +++
    PAVVKLVCG-DITVYETTVYISSILLLLPIFLISTSYVFILQSVIQMRSS 230

45  GPCR8    231 ...KAAKTLVVVVVFLCWLFPYFIVLLDLTLCLSIIMSSTCELERVLP
    ++++ ++ ++ +++++ +|+ ++ ++++ ++ +
    GSKRNAFATCGSHLTVVSLWFGACIFSVMRPRSQCT-----LL 268

    TALLVTLWLAYVNSCLNPIIY
    + +++++ + +++| +||

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The GPCR8 disclosed in this invention is expressed in at least the following tissues:

- 5 Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those
10 that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue
15 sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR8 is provided in Example 2.

- The nucleic acids and proteins of GPCR8 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related
20 pathological disorders, described further above.

- The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention
25 for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR8 Antibodies" section below. The disclosed GPCR8 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR8 epitope is from about amino acids 5 to 15. In another embodiment, a
30 GPCR8 epitope is from about amino acids 88 to 94. In further specific embodiments, GPCR8 epitopes are from about amino acids 130 to 135, from about amino acids 172 to 178, from about amino acids 220 to 235, from about amino acids 260 to 275 and from about amino acids 290 to 323.

GPCR9

The disclosed GPCR9 (also referred to as CG50203-01) includes the 932 nucleotide sequence (SEQ ID NO:73) shown in Table 9A. An open reading frame was identified beginning with an ACA which codes for the amino acid Threonine at nucleotides 3-5 and ending with a TGA codon at nucleotides 900-902. Putative untranslated regions, if any, are found upstream from the initiation codon and downstream from the termination codon and are underlined in Table 8A. The start and stop codons are in bold letters.

Table 9A. GPCR9 Nucleotide Sequence (SEQ ID NO:73)

TG**A**CAGAAATTCATTCTTCTTGGTCTGACTCAGTCTCAAGATGCTCAACTTCTGGTCTTTGTGCTAGT
CTTAATTTTCTACCTTATCATCCTCCCTGGAAATTTCTCATCATTTTACCATAAAGTCAGACCCT
GGGCTCACAGCCCCCTCTATTCTTTCTGGGCAACTTGGCCTTACTGGATGCATCCTACTCCTTCA
TTGTGGTTCCAGGATGTTGGTGGACTTCTCTCTGAGAAGAAGGTAATCTCCTATAGAAGCTGCAT
CACTCAGCTCTTTTCTTGCAATTTCTTGGAGCGGGAGAGATGTTCTCTCGTTGTGATGGCCTTT
GACCGCTACATCGCCATCTGCCGCCCTTTACACTATTCAACCATCATGAACCCTAGAGCCTGCTATG
CATTATCGTTGGTCTGTGGCTTGGGGGCTTTATCCATTCCATTGTACAAGTAGCCCTTATCCTGCA
CTTGCCCTTTCTGTGGCCCAACCAGCTCGATAACTTCTTCTGTGATGTTCCACAGGTTCATCAAGCTG
GCCTGCACCAATACCTTTGTGGTGGAGCTTCTGATGGTCTCCAACAGTGGCCTGCTCAGCCTCCTGT
GCTTCTGGGCCCTTCTGGCCTCCTATGCAGTCATCCTCTGTCTGTATAAGGGAGCACTCCTCTGAAGG
AAAGAGCAAGGCTATTTCCACATGCACCACCCATATTATCATTATATTTCTCATGTTTGGACCTGCT
ATTTTCATCTACACTTGCCCTTCCAGGCTTTCCAGCTGACAAGGTAGTTTCTCTTTTCCATACTG
TCATCTTTCTTTGATGAACCCGTGTATTTATACGCTTCGCAACCAGGAGGTGAAAGCTTCCATGAG
GAAGTTGTTAAGTCAACATATGTTTGTCTGAATAGAAGAAAGAGAAAAGCAAGAACGGAGA

The disclosed GPCR9 nucleic acid sequence of this invention has 550 of 848 bases (64%) identical to a *Mus musculus* gene for odorant receptor MOR10 (gb:GENBANK-ID:AB030893|acc:AB030893.1)($E = 5.4e-54$).

The GPCR9 protein (SEQ ID NO:74) encoded by SEQ ID NO:73 is 299 aa in length and is presented using the one-letter amino acid code in Table 9B. The Psort, SignalP and/or Hydropathy results predict that GPCR9 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. In alternative embodiments, a GPCR9 polypeptide is located to the Golgi body with a certainty of 0.4000, the endoplasmic reticulum (membrane) with a certainty of 0.3000, or the mitochondrial inner membrane with a certainty of 0.0300. The SignalP shows a signal sequence is coded for in the first 35 amino acids with a likely cleavage site at between positions 35 and 36, at NFL-II.

Table 9B. Encoded GPCR9 protein sequence (SEQ ID NO:74)

TEFILLGLTQSQDAQLLVFVLVLIIFYLIILPGNFLIIFTIKSDPGLTAPLYFFLGNLALLDASYSTI
VVPRMLVDFLSEKKVISYRSCITQLFFLHFLGAGEMFLLVMAFDRIYIAICRPLHYSTIMNPRACYA
LSLVWLWGGFIHSIVQVALILHLPFCGPNQLDNFFCDVPQVIKLACTNTFVVELLMVSNLSLLC

FLGLASYAVILCRIREHSSEGGKSAISTCTTHIIIFLMFGPAIFTYTCPPQAFPADKVVSLFHTV
IFPLMNPVIYTLRNQEVKASMRKLLSQHMF

The amino acid sequence of GPCR9 had high homology to other proteins as shown in Table 9C.

Table 9C. BLASTX results for GPCR9			
		High Score	Smallest Sum Prob P (N)
patp Sequences producing High-scoring Segment Pairs:			
AAG71733 Human olfactory receptor polypeptide, 308 aa		1536	1.6e-157
AA92364 Human G protein-coupled receptor protein 4, 307 aa		1336	2.6e-136
AAE02496 Human CON197 G protein-coupled receptor protein, 307 aa		1336	2.6e-136
AAG71483 Human olfactory receptor polypeptide, 317 aa		1335	3.3e-136
AAG71692 Human olfactory receptor polypeptide, 316 aa		1335	6.8e-136

GPCR9 also has homology to the proteins shown in the BLASTP data in Table 9D.

Table 9D. GPCR9 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Q9R0K3	ODORANT RECEPTOR MOR83 <i>Mus musculus</i>	308	163/299 (54%)	217/299 (72%)	3.9e-88
Q15615	Olfactory receptor 4D1 (Olfactory receptor TPCR16) - <i>Homo sapiens</i>	310	162/290 (55%)	216/290 (74%)	3.5e-87
P58180	Olfactory receptor 4D2 <i>Homo sapiens</i>	307	158/292 (54%)	217/292 (74%)	4.0e-86
Q9R0K4	ODORANT RECEPTOR MOR10 <i>Mus musculus</i>	310	154/293 (52%)	216/293 (73%)	2.8e-85
Q9R0K5	ODORANT RECEPTOR MOR28 <i>Mus musculus</i>	313	148/291 (50%)	210/291 (72%)	3.4e-82

A multiple sequence alignment is given in Table 9E, with the GPCR9 protein being shown on line 1, in a ClustalW analysis comparing GPCR9 with the related protein sequences disclosed in Table 9D.

Table 9E. ClustalW Analysis of GPCR9

- SEQ ID NO:74, GPCR9
- SEQ ID NO:75, Q9R0K3 ODORANT RECEPTOR MOR83
- SEQ ID NO:76, Q15615 Olfactory receptor 4D1
- SEQ ID NO:77, P58180 Olfactory receptor 4D2
- SEQ ID NO:78, Q9R0K4 ODORANT RECEPTOR MOR10
- SEQ ID NO:79, Q9R0K5 ODORANT RECEPTOR MOR28

10 20 30 40 50

P58180 VRRIGRHRLV---
Q9ROK4 LNKLIKRRREK---
Q9ROK5 LKKLIRKKEGKEK

5

Table 9F lists the domain description from DOMAIN analysis results against GPCR9. This indicates that the GPCR9 sequence has properties similar to those of other proteins known to contain this domain as well as to the 254 amino acid 7tm domain (SEQ ID NO:18) itself.

10

Table 9F Domain Analysis of GPCR9

PSSMs producing significant alignments:			Score	E
			(bits)	value
gnl Pfam pfam00001	7tm_1, 7 transmembrane receptor (rhodopsin family)		110.9	3.1e-34
7tm_1		GNLLVILVILRTKKLRTPTNIFILNLAVADLLFLLTLPPWALYYLVG +++++ + + +++++ + +++ +++ +++ ++		
GPCR9	32	GNFLIIFTIKSDPGLTAPLYFFLGNLALLDASYSFIVVPRMLVDFLS	78	
		GSEDWPFGSALCKLVLTALDVVNMYASILLTASIDRYLAIVHPLRYRRR + +++++ ++ ++ + + +++ ++++ + ++ +++++		
GPCR9	79	--EKKVISYRSCITQLFFLHFLGAGEMFLLVMAFDRIYIAICRPLHYSTI	126	
		RTSPRAKVVILLVWVLALLLSLPPLLFSWVKTVEEGNGTLN..... ++ ++ ++++++ + +++ ++ ++ + ++++++ +++++ + ++		
GPCR9	127	MN-PRACYALSLVLWLGGFIHSIVQVAL-ILHLPFCGPNQLDnffcdvpq	174	
		VNVTVCLIDFPEESTASVSTWLRSYVLLSTLVGFLLPLLVLVCYTRILR +++ + + +++ + + ++ + + +++++ ++ + ++		
GPCR9	175	VIKLACTNTFVVEL-----LMVSNSSLLSLLCFLGLLASYAVILC	214	
		TLR.....KAAKTLVVVVVFLCWLPHYFIVLLD TLC.LSIIMSSTC +++++++ + + ++ ++ ++ + +++++ ++ ++		
GPCR9	215	RIRhssegkSKAISTCTTHIIIFLMFGPAIFIYTCPPQaFP-----	257	
		ELERVLPTALLVTLWLAYVNSCLNPIIY +++ +++++ + + + + +		
GPCR9	258	-----ADKVVSLFHTVIF--PLMNPVIY	278	

40

The GPCR9 disclosed in this invention is expressed in at least the following tissues:
Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain,
Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus,
CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells,
endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye,

frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR9 is provided in Example 2.

In addition, the sequence is predicted to be expressed in the following tissues because of the expression pattern of (GENBANK-ID: gb:GENBANK-ID:AB030893|acc:AB030893.1) a closely related *Mus musculus* gene for odorant receptor MOR10, complete cds homolog in species *Mus musculus*: Brain.

The nucleic acids and proteins of GPCR9 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further above.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. The disclosed GPCR9 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR9 epitope is from about amino acids 75 to 80. In another embodiment, a GPCR9 epitope is from about amino acids 118 to 123. In further specific embodiments, GPCR9 epitopes are from about amino acids 127 to 132, from about amino acids 165 to 172, from about amino acids 215 to 230 and from about amino acids 280 to 299.

GPCR10

GPCR10 includes two GPCR proteins disclosed below. The disclosed proteins have been named GPCR10a and GPCR10b, and are related to olfactory receptors.

GPCR10a

The disclosed GPCR10a nucleic acid (SEQ ID NO:80) of 984 nucleotides (also referred to as CG50197-01) is shown in Table 10A. An open reading frame was identified beginning with an CTC initiation codon, which codes for leucine, at nucleotides 2-4 and ending with a TAA codon at nucleotides 941-943. Putative untranslated regions found upstream from the first codon and downstream from the termination codon are underlined in Table 10A, and the start and stop codons are in bold letters.

Table 10A. GPCR10a Nucleotide Sequence (SEQ ID NO:80)

CCTCCAAAGAGCCACTTTCTTCCTGACGGGCTTCCAAGGTCTAGAAGGTCTCCATGGCTGGATCTCT
ATTCCCTTCTGCTTCATCTACCTGACAGTTATCTTGGGGAACCTCACCATTCTCCACGTCATTTGTA
CTGATGCCACTCTCCATGGACCCATGTACTATTTCTTGGGCATGCTAGCTGTCACAGACTTAGGCCT
TTGCCTTTCCACACTGCCACTGTGCTGGGCATTTTCTGGTTTGATAACCAGAGAGATTGGCATCCCT
GCCTGTTTCACTCAGCTCTTCTTCATCCACACCTTGTCTTCAATGGAGTCATCAGTTCTGTTATCCA
TGTCCATTGACCGCTACGTGGCCGTCTGCAACCCACTGCATGACTCCACCGTCCTGACACCTGCATG
TATTGTCAAGATGGGGCTAAGCTCAGTGCTTAGAAGTGCTCTCCTCATCCTCCCCCTTGCCATTCTC
CTGAAGCGCTTCCAATACTGCCACTCCCATGTGCTGGCTCATGCTTATTGTCTTCACCTGGAGATCA
TGAAGCTGGCCTGCTCTAGCATCATTGTCAATCACATCTATGGGCTCTTTGTTGTGGCCTGCACCGT
GGGTGTGGACTCCCTGCTCATCTTTCTCTCATACGCCCTCATCCTTCGCACCGTGCTCAGCATTGCC
TCCCACCAGGAGCGACTCCGAGCCCTCAACACCTGTGTCTCTCATATCTGTGCTGTACTGCTCTTCT
ACATCCCCATGATTGGCTTGTCTCTTGTGCATCGCTTTGGTGAACATCTGCCCCGCGTTGTACACCT
CTTCATGTCTATGTGTATCTGCTGGTACCACCCCTTATGAACCCATCATCTACAGCATCAAGACC
AAGCAAATTCGCCAGCGCATCATTAGAAGTTTCAGTTTATAAAGTCACTTAGGTGTTTTTGAAGG
ATTAAGTTAGAGTAAAGAGAGGAAGTTTTGGACATAAAGCCACAG

The disclosed GPCR10a nucleic acid sequence has 593 of 888 bases (66%) identical to a *Mus musculus* MOR 5'beta3, MOR 5'beta2, and MOR 5'beta1 mRNA; beta-globin (Hbb) gene, Hbb-D allele, locus control region (gb:GENBANK-ID:AF071080|acc:AF071080.2) (E = 1.1e⁻⁶¹).

The disclosed GPCR10a polypeptide (SEQ ID NO:81) encoded by SEQ ID NO:80 has 313 amino acid residues and is presented using the one-letter amino acid code in Table 10B. The SignalP, Psort and/or Hydropathy results predict that GPCR10a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a GPCR10a peptide is between amino acids 38 and 39, at: NTL-IL.

Table 10B. GPCR10a protein sequence (SEQ ID NO:81)

LQRATFFLTGFQGLEGLHGWISIPFCFIYLTIVILGNLTILHVICTDATLHGPMYYFLGMLAVTDLGLCL

STLPTVLGIFWFDTREIGIPACFTQLFFIHTLSSMESSVLLSMSIDRYVAVCNPLHDSTVLTPACIVKM
 GLSSVLRSAALLILPLPFLKRFQYCHSHVLAHAYCLHLEIMKLACSSIIIVNHIYGLFVVACTVGVDLL
 IFLSYALILRTVLSIASHQERLRALNTCVSHICAVLLFYIPMIGLSLVHRFGEHLPRVVHLFMSYVYLL
 VPPLMNPPIIYSIKTKQIRQRIKKFQFIKSLRCFWKD

The disclosed GPCR10a amino acid sequence has 161 of 301 amino acid residues (53%) identical to, and 223 of 301 amino acid residues (74%) similar to, the *Mus musculus* 315 amino acid residue MOR 5'BETA3 protein (ptnr:SPTREMBL-ACC:Q9WVN6)(E = 7.2e⁻⁸⁹).

GPCR10b

In the present invention, the target sequence identified previously, Accession Number CG50197-01, was subjected to the exon linking process to confirm the sequence. GPCR10b was isolated as described in Example 1. GPCR10b differs from the previously identified sequence (Accession Number CG50197-01) in having 2 extra amino acid at N-terminal and 1 amino acid changed at position 117 Y->S.

The disclosed GPCR10b nucleic acid (SEQ ID NO:82) of 1008 nucleotides (also referred to as CG50197-02) is shown in Table 10C. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 20-22 and ending with a TAA codon at nucleotides 965-967. Putative untranslated regions found upstream from the initiation codon and downstream from the termination codon are underlined in Table 10C, and the start and stop codons are in bold letters.

Table 10C. GPCR10b Nucleotide Sequence (SEQ ID NO:82).

CTATGACAATTCTTCTTAAT**GCAGCCTCCAAAGAGCCACTTTCTTCCTGACGGGCTTCCAAGGTCTAGAA**
GGTCTCCATGGCTGGATCTCTATCCCTTCTGCTTCATCTACCTGACAGTTATCTTGGGGAACCTCACCA
 TTCTCCACGTCATTTGTACTGATGCCACTCTCCATGGACCCATGTACTATTTCTTGGGCATGCTAGCTGT
 CACAGACTTAGGCCTTTGCCTTTCCACACTGCCACTGTGCTGGGCATTTTCTGGTTTGATACCAGAGAG
 ATTGGCATCCCTGCCTGTTTCACTCAGCTCTTCTTCATCCACACCTTGTCTTCAATGGAGTCATCAGTTC
 TGTATCCATGTCCATTGACCGCTCCGTGGCCGTCTGCAACCCACTGCATGACTCCACCGTCTTGACACC
 TGCATGTATTGTCAAGATGGGGCTAAGCTCAGTGCTTAGAAGTGCTCTCCTCATCCTCCCCTTGCCATTCT
 CTCCTGAAGCGCTTCCAATACTGCCACTCCCATGTGCTGGCTCATGCTTATTGTCTTCACTGGAGATCA
 TGAAGCTGGCCTGCTCTAGCATCATTTGTCAATCACATCTATGGGCTCTTTGTTGTGGCCTGCACCGTGGG
 TGTGGACTCCCTGCTCATCTTTCTCTCATACGCCCTCATCCTTCGCACCGTGCTCAGCATTGCCTCCCA
 CAGGAGCGACTCCGAGCCCTCAACACCTGTGTCTCTCATATCTGTGCTGTACTGCTCTTCTACATCCCCA
 TGATTGGCTTGTCTCTTGTGCATCGCTTTGGTGAACATCTGCCCCGCGTTGTACACCTCTTCATGTCCTA
 TGTGTATCTGCTGGTACCACCCCTTATGAACCCCATCATCTACAGCATCAAGACCAAGCAAATTCGCCAG
 CGCATCATTAAGAAGTTTCAGTTTATAAAGTCACTTAGGTGTTTTTGAAGGAT**TAA**GTAGAGTAAAGA
GAGGAAGTTTTGGACATAAAGCCCACAG

The disclosed GPCR10b nucleic acid sequence has 564 of 880 bases (64%) identical to a *Mus musculus* odorant receptor S19 mRNA (gb:GENBANK-ID:AF121976|acc:AF121976.2) ($E = 1.9e^{-51}$).

The disclosed GPCR10b polypeptide (SEQ ID NO:83) encoded by SEQ ID NO:82 has 315 amino acid residues and is presented using the one-letter amino acid code in Table 10D. The SignalP, Psort and/or Hydropathy results predict that GPCR10b has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a GPCR10b peptide is between amino acids 49 and 50, at: TDA-TL.

Table 10D. GPCR10b protein sequence (SEQ ID NO:83)

CSLQRATFFLTGFQGLEGLHGWISIPFCFIYLTIVILGNLTILHVICTDATLHGPMYYFLGMLAVTDLGL CLSTLPTVLGIFWFDTREIGIPACFTQLFFIHTLSSMESSVLLSMSIDRSVAVCNPLHDSVTLPACIV KMGLSSVLRSAALLILPLPFLKRFQYCHSHVLAHAYCLHLEIMKLACSSIIIVNHIYGLFVVACTVGVD LLIIFLSYALILRTVLSIASHQERLRALNTCVSHICAVLLFYIPMIGLSLVHRFGEHLPRVVHLFMSYV LLVPPLMNPPIIYSIKTKQIRQRIKKFQFIKSLRCFWKD

The disclosed GPCR10b amino acid sequence 164 of 295 amino acid residues (55%) identical to, and 223 of 295 amino acid residues (75%) similar to, the *Mus musculus* 319 amino acid residue protein; MOR 3'BETA4 (ptnr:TREMBLNEW-ACC:AAG41684)($E = 8.1e^{-90}$).

GPCR10 Family

The term GPCR10 is used to refer to all GPCR10 variants or members of the GPCR10 family disclosed herein unless we identify a specific family member or variant.

GPCR10 is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus

tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR10 is provided in Example 2.

In addition, the GPCR10 sequence is predicted to be expressed in brain tissue because of the expression pattern of a closely related *Mus musculus* MOR 5'beta3, MOR 5'beta2, and MOR 5'beta1 genes, complete cds; beta-globin (*Hbb*) gene, *Hbb-D* allele, locus control region homolog (GENBANK-ID: gb:GENBANK-ID:AF071080|acc:AF071080.2).

Homologies between the GPCR10 variants is shown in a Clustal W in Table 10F.

Table 10F. Clustal W of GPCR10 Family

		10	20	30	40	50	60	
15	GPCR10a	CCTCCAAGAGCCACTTTCTTCTCTGACGGGCTTCCA					
	GPCR10b	CTATGACAATTCTTCTTAATGCAG	CCTCCAAGAGCCACTTTCTTCTCTGACGGGCTTCCA					
		70	80	90	100	110	120	
20	GPCR10a	AGGTCTAGAAGGTCTCCATGGCTGGATCTCTATTCCCTTCTGCTTCATCTACCTGACAGT						
	GPCR10b	AGGTCTAGAAGGTCTCCATGGCTGGATCTCTATTCCCTTCTGCTTCATCTACCTGACAGT						
		130	140	150	160	170	180	
25	GPCR10a	TATCTTGGGGAACCTCACCATTCTCCACGTCATTTGTACTGATGCCACTCTCCATGGACC						
	GPCR10b	TATCTTGGGGAACCTCACCATTCTCCACGTCATTTGTACTGATGCCACTCTCCATGGACC						
		190	200	210	220	230	240	
30	GPCR10a	CATGTACTATTCTTGGGCATGCTAGCTGTCAACAGACTTAGGCCTTTGCCTTTCCACACT						
	GPCR10b	CATGTACTATTCTTGGGCATGCTAGCTGTCAACAGACTTAGGCCTTTGCCTTTCCACACT						
		250	260	270	280	290	300	
35	GPCR10a	GCCCACTGTGCTGGGCATTTTCTGGTTTGATACCAGAGAGATTGGCATCCCTGCCTGTTT						
	GPCR10b	GCCCACTGTGCTGGGCATTTTCTGGTTTGATACCAGAGAGATTGGCATCCCTGCCTGTTT						
		310	320	330	340	350	360	
40	GPCR10a	CACTCAGCTCTTCTTCATCCACACCTTGTCTTCAATGGAGTCATCAGTTCTGTTATCCAT						
	GPCR10b	CACTCAGCTCTTCTTCATCCACACCTTGTCTTCAATGGAGTCATCAGTTCTGTTATCCAT						
		370	380	390	400	410	420	
45	GPCR10a	GTCCATTGACCGCTACGTTGGCCGTCTGCAACCCACTGCATGACTCCACCGTCTGACACC						
	GPCR10b	GTCCATTGACCGCTACGTTGGCCGTCTGCAACCCACTGCATGACTCCACCGTCTGACACC						
		430	440	450	460	470	480	
50	GPCR10a	TGCATGTATTGTCAAGATGGGGCTAAGCTCAGTGCTTAGAAGTGCTCTCCTCATCTCCG						
	GPCR10b	TGCATGTATTGTCAAGATGGGGCTAAGCTCAGTGCTTAGAAGTGCTCTCCTCATCTCCG						
		490	500	510	520	530	540	
55	GPCR10a	CTTGCCATTCTCTGAAGCGCTTCCAATACTGCCACTCCCATGTGCTGGCTCATGCTTA						
	GPCR10b	CTTGCCATTCTCTGAAGCGCTTCCAATACTGCCACTCCCATGTGCTGGCTCATGCTTA						
		550	560	570	580	590	600	

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GPCR10a
GPCR10b

610 620 630 640 650 660

670 680 690 700 710 720

730 740 750 760 770 780

790 800 810 820 830 840

850 860 870 880 890 900

910 920 930 940 950 960

970 980 990 1000

TTGTCCTTCACCTGGAGATCATGAAGCTGGCCTGCTCTAGCATCATTGTCAATCACATCTA
TTGTCCTTCACCTGGAGATCATGAAGCTGGCCTGCTCTAGCATCATTGTCAATCACATCTA

TGGGCTCTTTGTTGTGGCCTGCACCGTGGGTGTGGACTCCCTGCTCATCTTTCTCTCATA
TGGGCTCTTTGTTGTGGCCTGCACCGTGGGTGTGGACTCCCTGCTCATCTTTCTCTCATA

CGCCCTCATCTTCGCACCGTGTCTCAGCATTGCCTCCACCAGGAGCGACTCCGAGCCCT
CGCCCTCATCTTCGCACCGTGTCTCAGCATTGCCTCCACCAGGAGCGACTCCGAGCCCT

CAACACCTGTGCTCTCATATCTGTGCTGTACTGCTCTTCTACATCCCCATGATTGGCTT
CAACACCTGTGCTCTCATATCTGTGCTGTACTGCTCTTCTACATCCCCATGATTGGCTT

GTCTCTGTGTCATCGCTTTGGTGAACATCTGCCCGCGCTTGTACACCTCTTCATGTCCTA
GTCTCTGTGTCATCGCTTTGGTGAACATCTGCCCGCGCTTGTACACCTCTTCATGTCCTA

TGTGTATCTGCTGGTACCACCCCTTATGAACCCCATCATCTACAGCATCAAGACCAAGCA
TGTGTATCTGCTGGTACCACCCCTTATGAACCCCATCATCTACAGCATCAAGACCAAGCA

AATTGCCAGCGCATCATTAAGAAGTTTCAGTTTATAAAGTCACTTAGGTGTTTTTGGAA
AATTGCCAGCGCATCATTAAGAAGTTTCAGTTTATAAAGTCACTTAGGTGTTTTTGGAA

GGATTAAGTTAGAGTAAAGAGAGGAAGTTTGGACATAAAGCCACAG
GGATTAAGTTAGAGTAAAGAGAGGAAGTTTGGACATAAAGCCACAG

The disclosed GPCR10a has homology to the amino acid sequences shown in the BLASTP data listed in Table 10G.

Table 10G. BLASTP results for GPCR10a

Gene Index/Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 11908218 gb AAG41683.1 (AF137396)	HOR5'Beta5 [Homo sapiens]	312	149/282 (52%)	194/282 (67%)	2e-68
gi 11908220 gb AAG41684.1 (AF133300)	MOR 3'Beta4 [Mus musculus]	319	145/284 (51%)	197/284 (69%)	5e-68
gi 7305343 ref NP_038644.1	olfactory receptor 64 [Mus musculus]	315	142/287 (49%)	195/287 (67%)	1e-67
gi 7305347 ref NP_038646.1	olfactory receptor 66 [Mus musculus]	311	148/284 (52%)	196/284 (68%)	2e-67
gi 7305345 ref NP_038645.1	olfactory receptor 65 [Mus musculus]	307	149/287 (51%)	194/287 (66%)	2e-67

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 10H.

Table 10H. ClustalW Analysis of GPCR10a

5	1) GPCR10a (SEQ ID NO:81)
	2) AF137396 (SEQ ID NO:84)
	3) AF133300 (SEQ ID NO:85)
10	4) NP_038644.1 (SEQ ID NO:86)
	5) NP_038646.1 (SEQ ID NO:87)
	6) NP_038645.1 (SEQ ID NO:88)
15	<div> <div> 102030405060 </div> <div> <div>GPCR10a</div> <div>gi 11908218</div> <div>gi 11908220</div> <div>gi 7305343</div> <div>gi 7305347</div> <div>gi 7305345</div> </div> <div> <div>-LQRTATFELTGFGQGLEGLHGWISIPPCFFVETVHGNLTLLHVICTDASLHGF </div> <div> -----MSSSSSHPPFLLTGFPGLEEAHHWISVFELFMYHSLIFGNSTLLLLIKEDHNLHEP </div> <div> MATSNSSTFVSSFTFYLTGIPGYDEPHHWISIPPCFFVETVGTTCNCMTTHHATDPRLHEP </div> <div> -MPSSWLNLSSSPFLLTGFPGLEKAHHLISPELLMAYHSLILGNGTLLFLIKDDHNLHEP </div> <div> ---MWPNSDAPFLLTGFLGLEMIHHWISIPHFVLYESLIVGNGTLLFTHWSDHSLHEP </div> <div> ----MWSNLSAPFLLTGFPGLEAAHHWISIPFATYHSLVGLNGTLLYLIKDDHNLHEP </div> </div> </div>
20	<div> <div> 708090100110120 </div> <div> <div>GPCR10a</div> <div>gi 11908218</div> <div>gi 11908220</div> <div>gi 7305343</div> <div>gi 7305347</div> <div>gi 7305345</div> </div> <div> <div> MYYFLGLAVTDLGLCTLTPTVLGTFWEDTRETGIPACFTCLEFIHQLSSMESSVLLSM </div> <div> MYFLAMLAATDLGALTTMPTVIGVLWEDHREIGSAACFSCAYFIHSLSLFESGVLLAM </div> <div> MYYFLAMLSLTDVANSLETMMSLFRVLWSISRETQFNICVVMGLIHETFTESSVLLAM </div> <div> MYYFLGLAVTDLGLCTLTMPTVLSVLWLNHREIGHGACFSCAYFIHSLSLVESGVLLAM </div> <div> MYYFLAVLASMDLGLTMTPTVIGVLVNLQREIVHGACFTCSYFIHSLSLVESGVLLAM </div> <div> MYYFLAMLAATDLGLTMTPTVMVAVLWLNHREIRHACFTQAYTIHSLSLVESGVLLAM </div> </div> </div>
30	<div> <div> 130140150160170180 </div> <div> <div>GPCR10a</div> <div>gi 11908218</div> <div>gi 11908220</div> <div>gi 7305343</div> <div>gi 7305347</div> <div>gi 7305345</div> </div> <div> <div> SYDRFVAICNPILHDSYLTLPACTVKGCLSSVLSAALLTLPFLPKRFOYCHSHVLASHAF </div> <div> AYDRFVAICNPILRSTSLTNRVVKIGLQVLMRGFVSAPPFIRPTLYLYCHSHVLSHAF </div> <div> AYDRFVAICNPILRVATLTPEKLTAKIGTAALLRSSLTLPILARLAPFCGSHVLSHST </div> <div> AYDRFVAICNPILRYTLLTDITKVKIGLGLVMRAGLSIMPEIIRHWHFECRSHVLSHAF </div> <div> SYDRFVAICTPLEMNSILTNSRVKVALGALLRGFVSIVPEPIMPFWFECYCHSHVLSHAF </div> <div> SYDRFVAICTPLEMNSILTNSFVLAIGLQVLMRGFVSIVPEPILPFWFESYCRSHVLSHAF </div> </div> </div>
40	<div> <div> 190200210220230240 </div> <div> <div>GPCR10a</div> <div>gi 11908218</div> <div>gi 11908220</div> <div>gi 7305343</div> <div>gi 7305347</div> <div>gi 7305345</div> </div> <div> <div> CLHQLRMLKLACSTIIVNHIYCHFTVACTVGDLSLTHLSYALITLTVESTASHGERLAL </div> <div> CLHQDVVKLACADITFNRIYEAFLVVFIFVLVDYLIIFTSYVLILKTVLSIASREERAKAL </div> <div> CLHQDVVKLACADITFNRIYGVVLTLLGMDSLGIEVSVYLILHSVKIASREGRLKAL </div> <div> CLHQDVVKLACADITFNRIYPPVVVFAMVLLDGLIIFSYVLILKTVMGIASTEERAKAL </div> <div> CLHQDVVKLACADITFNRIYPPVVLVALTFELDGLIIFSYVLILKTVMGIASTEERAKAL </div> <div> CLHQDVVKLACADITFNRIYPPVVLVALTFELDGLIIFSYVLILKTVMGIASTEERAKAL </div> </div> </div>
50	<div> <div> 250260270280290300 </div> <div> <div>GPCR10a</div> <div>gi 11908218</div> <div>gi 11908220</div> <div>gi 7305343</div> <div>gi 7305347</div> <div>gi 7305345</div> </div> <div> <div> NTCVSHICAVLLEFYTPMIGLSLHFRFGKELPRVVHGFMSYNYLWPPPLMNPPIYSIKTKQ </div> <div> ITCVSHICCVLVFYTVVIGLSLHFRFGKQVPHVHLIMSAYFLEPPPLMNPPIYSIKTKQ </div> <div> NTCASHICAVLLEFYTPMIGLSLHFRFAKHSSPLHIFMAHLYLWPPVLPNPPIYSIKTKQ </div> <div> NTCVSHICCLLVFYTVVIGLSLHFRFGKNVPHVVHITMSYNYFLFPPPMNPPIYSIKTKQ </div> <div> NTCVSHISCVLVFYTVVIGLSLHFRFGKQAPHVVHITMSYNYFLFPPPMNPPIYSIKTKQ </div> <div> NTCVSHISCVLVFYTVVIGLSLHFRFGKQAPHVVHITMSYNYFLFPPPMNPPIYSIKTKQ </div> </div> </div>
60	<div> <div> 310320 </div> <div> <div>GPCR10a</div> <div>gi 11908218</div> <div>gi 11908220</div> <div>gi 7305343</div> <div>gi 7305347</div> <div>gi 7305345</div> </div> <div> <div> TRORTLKKFQFIKSLRCFWKD </div> <div> IQNALDHLFTTHRIGT----- </div> <div> IREGILHLCSPKISSITM-- </div> <div> IQSGELRLFSLPCKSKT----- </div> <div> IQRSILRLLSKHSRT----- </div> <div> IQRSYHLHLSV----- </div> </div> </div>
65	

The homologies shown above are shared by GPCR10b insofar as GPCR10a and GPCR10b are homologous as shown in Table 10F.

Table 10I lists the domain description from DOMAIN analysis results against GPCR10a. This indicates that the GPCR10a sequence has properties similar to those of other proteins known to contain this domain as well as to the 377 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 10I. Domain Analysis of GPCR10a

gnl|Pfam|pfam00001, 7tm_1, 7 transmembrane receptor
(rhodopsin family). (SEQ ID NO:XXX)
Length = 254 residues, 100.0% aligned
Score = 58.5 bits (140), Expect = 5e-10

10	GPCR10a 35	GNLTILHVICTDATLHGPMYYFLGMLAVTDLGLCLSTLPTVLGIFWFDTREIGIPACFTQ	94
	00001 1	GNLLVILVILRTKKLRTPTNIFLLNLAVADLLFLLTLPWALYYLVGGDWVFGDALCKLV	60
15	GPCR10a 95	LFFIHTLSSMESSVLLSMSIDRYVAVCNPLHDSTVLTPACIVKMGLSSVLRSA--LLILP	152
	00001 61	GALFVVNGYASILLTASIDRYLAIVHPLRYRRIRTPRRAKVLILLVWVLALLLSLPPL	120
20	GPCR10a 153	LPFLLKRFQYCHSHVLAHAYCLHLEIMKLACSSIIVNHIYGLFVVACTVGVDSELLIFLSY	212
	00001 121	LFSWLRTVEEGNTTVCLIDFPEESVKRSYVLLSTLVGFVLPPLLVILVCYTR--ILRTLK	178
25	GPCR10a 213	ALILRTVLSIASHQERLRALNTCVSHICAVLLFYIPMIGLSLVHRFGEHLPRVVHLFMSY	272
	00001 179	RARSQRLKRRSSSER-KAAKMLLVVVVVFVLCWLPYHIVLLDLSLCLLSIWRVLPPTALL	237
30	GPCR10a 273	VYLLVP---PLMNPIIY	286
	00001 238	ITLWLAYVNSCLNPIIY	254

GPCR10 polypeptides are useful in the generation of antibodies that bind immunospecifically to the GPCR10 polypeptides of the invention. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR10 Antibodies" section below. The disclosed GPCR10 proteins have multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR10 epitope is from about amino acids 225 to 235. In another embodiment, a GPCR10 epitope is from about amino acids 280 to 305. The GPCR10 proteins also have value in the development of powerful assay system for functional analysis

of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

GPCR11

5 The disclosed GPCR11 nucleic acid (SEQ ID NO:89) of 922 nucleotides (also referred to as CG50199-01) is shown in Table 11A . An open reading frame was identified beginning with an AGT initiation codon, which codes for serine, at nucleotides 2-4 and ending with a TGA codon at nucleotides 920-922. A putative untranslated region upstream from the initiation codon is underlined in Table 11A, and the start and stop codons are in bold letters.

Table 11A. GPCR11 Nucleotide Sequence (SEQ ID NO:89)

CAGTGAATTTGTTCTCGTGAGCTTCTCAGCCCTGTCCACTGAGCTTCAGGCTCTACTGTTTCTCCTTTT
CTTGACCATTTTACTTGGTTACTTTAATGGGCAATGTCTCATCATCCTGGTCACATAGCTGACTCTGC
ACTACAAAGTCTATGTACTTCTTCCCTCAGAACTTGTCTTCTGGAGATAGGTTTCAACTTGGTCAT
TGTGTCCAAGATGCTGGGGACCCTGATCATTCAAGACACAACCATCTCCTTCTTGGATGTGCCACTCA
GATGATTTTCTTCTCTTTTTTGGGGCTGCTGAGTGCTGCCTCCTGGCCACCATGGCATATGACCGCTA
CGTGGCCATCTGTGACCCCTTGTACTACCCAGTCATCATGGGCCACATATCCTGTGCCCAGCTGGCAGC
TGCCTCTTGGTTCTCAGGGTTTTTCAGTGGCCACTGTGCAAACCACATGGATTTTTCAGTTTCCCTTTTTG
TGGCCCCAACAGGGTGAACCACTTCTTCTGTGACAGCCCTCCTGTTATTGCACTGGTCTGTGCTGACAC
CTCTGTGTTTGAACCTGGAGGCTCTGACAGCCACTGTCTTATTCTCTTTCTTTCTTCTGCTGATCCT
GGGATCCTATGTCCGCATCCTCTCCACTATCTTCTCAGGATGCCGTCAGCTGAGGGGAAACATCAGGCATT
CTCCACCTGTTCCGCCACCTCTTGGTTGTCTCTCTTCTATAGCACTGCCATCCTCAGTATTTCCG
ACCCCAATCCAGTGCCTCTTCTGAGAGCAAGAAGCTGCTGTCACTCTCTTCCACAGTGGTGACTCCCAT
GTTGAACCCCATCATCTACAGCTCAAGGAATAAGAAGTGAAGGCTGCACTGAAGCGGCTTATCCACAG
GAACCTGGGCTCTCAGAACTATGA

10 The disclosed GPCR11 nucleic acid sequence of this invention has 794 of 922 bases (86%) identical to a *Mus musculus* olfactory receptor P2 (Olfr17) mRNA (gb:GENBANK-ID:AF247657|acc:AF247657.1) ($E = 1.7e^{-149}$).

15 The disclosed GPCR11 polypeptide (SEQ ID NO:90) encoded by SEQ ID NO:89 has 306 amino acid residues and is presented using the one-letter code in Table 11B. The SignalP, Psort and/or Hydropathy results predict that GPCR11 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The most likely cleavage site for a GPCR11 peptide is between amino acids 45 and 46, at: ADS-AL.

Table 11B. Encoded GPCR11 protein sequence (SEQ ID NO:90).

SEFVLVSFSALSTELQALLFLFLFTIYLVTLMGNVLIILVTIADSALQSPMYFFLRNLSFLEIGFNLVI
VSKMLGTLIIQDTTISFLGCATQMYFFFFGAAECCLLATMAYDRYVAICDPLYYPVIMGHISCAQLAA
ASWFSGFSVATVQTTWIFSFPFCGPNRVNHFFCDSPPVIALVCADTSVFELEALTATVLFILFPFLLIL

GSYVRILSTIFRMPSAEGKHQAFSTCSAHLVVSIFYSTAILTYFRPQSSASSESKKLLSLSTVVTMP
LNPIIYSSRNKEVKAALKRLIHRNLGSQKL

The disclosed GPCR11 amino acid sequence has 268 of 305 amino acid residues (87%) identical to, and 286 of 305 amino acid residues (93%) similar to, the *Mus musculus* 315 amino acid residue protein olfactory receptor P2 (ptnr:SPTREMBL-ACC:Q9JKA6) ($E = 8.6e^{-141}$).

GPCR11 is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, Those that express MHC II and III nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR11 is provided in Example 2.

In addition, the GPCR11 sequence is predicted to be expressed in brain tissue because of the expression pattern of a closely related *Mus musculus* olfactory receptor P2 (Olfr17) gene homolog (GENBANK-ID: gb:GENBANK-ID:AF247657|acc:AF247657.1).

The disclosed GPCR11 has homology to the amino acid sequences shown in the BLASTP data listed in Table 11C.

Table 11C. BLASTP results for GPCR11						
Gene Index/Identifier		Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 14423806 sp Q9H209 OAA4_HUMAN		OLFACTORY RECEPTOR 10A4 (HP2) [Homo sapiens]	315	256/306 (83%)	257/306 (83%)	1e-122
gi 10181106 ref NP_065623.1		olfactory receptor 17 [Mus musculus]	315	230/305 (75%)	244/305 (79%)	1e-112

gi 10181106	DSPPVIALVCADTSIFLEALTATVLFILFPFLLILGSYVRILSTIFFMPSAEGKRKAFS
gi 14423805	DSPPVILKVCADTALFEIYAIVGTILVVMIPOLLILCSYTRIAAAILKIPSAKGKHKAFS
gi 12007412	DSPPVLRVCADTAQFEVYAIVGTILVVMIPOLLILCSYTLIAAAILKIPSAKGKHKAFS
gi 12007437	DSPPVLRVCADTALFEIYAIVGTILVVMIPOLLILCSYTHIAAAILKIPSAKGKHKAFS
5	
	250 260 270 280 290 300
GPCR11	TCSSHLLVVSIFYSTAILTYFRPQSSASSESKKLLSLSTVVTPMLNPPIIYSSRNKEVKA
gi 14423806	TCSSHLLVVSIFYSTAILTYFRPQSSASSESKKLLSLSTVVTPMLNPPIIYSSRNKEVKA
10	
gi 10181106	TCSSHLLVVSIFYSTAILTYFRPQSSASSESKKLLSLSTVVTPMLNPPIIYSSRNKEVKA
gi 14423805	TCSSHLLVVSIFYSTAILTYFRPQSSASSESKKLLSLSTVVTPMLNPPIIYSSRNKEVKA
gi 12007412	TCSSHLLVVSIFYSTAILTYFRPQSSASSESKKLLSLSTVVTPMLNPPIIYSSRNKEVKA
gi 12007437	TCSSHLLVVSIFYSTAILTYFRPQSSASSESKKLLSLSTVVTPMLNPPIIYSSRNKEVKA
15	
	310
GPCR11	ALKRLIHRNLGSOKL--
gi 14423806	ALKRLIHRNLGSOKL--
gi 10181106	ALKRLIHRNLGSOKL--
20	
gi 14423805	ALSRTFHKLALALNCIP
gi 12007412	ALSRTFHKLALALNCIP
gi 12007437	ALSRTFHKLALALNCIP

Table 11E lists the domain description from DOMAIN analysis results against GPCR11. This indicates that the GPCR11 sequence has properties similar to those of other proteins known to contain this domain as well as to the 377 amino acid 7tm domain (SEQ ID NO:18) itself.

Table 11E. Domain Analysis of GPCR11	
gnl Pfam pfam00001, 7tm_1, 7 transmembrane receptor (rhodopsin family). (SEQ ID NO:XXX)	
Length = 254 residues, 100.0% aligned	
Score = 85.5 bits (210), Expect = 4e-18	

GPCR11	33	GNVLIILVTIADSALQSPMYFFLRNLSFLEIGFNLVIVSKMLGTLIIQDTTISFLGCATQ	92
00001	1	GNLLVILVILRTKKLRTPTNIFLLNLAVADLLFLLTLPPWALYYLVGGDWVFGDALCKLV	60
35			
GPCR11	93	MYFFFFFGAAECCLLATMAYDRYVAICDPLYYPVIMGHISCAQLAAASWFGFSVATVQT	152
00001	61	GALFVVNGYASILLTALSIDRYLAIVHPLRYRRIRTPRRAKVLILLVWVLAALLSLPPL	120
40			
GPCR11	153	TWIFSFPFCGPNRVNHFFCDSPPVIALVCADTSVFELEALTATVLFILFPFLLILGSYVR	212
00001	121	LFSWLRTVEEGNTTVCLIDFPEESVKRSYVLLSTLVGFVLPPLVILVCYTRILRTLKRA	180
45			
GPCR11	213	ILSTIFRMPSPAEGKHQAFSTCSAHLLVVSIFYSTAIL---TYFRPQSSASSESKKLLSL	268
00001	181	RSQRSLKRRSSSERKAAKMLLVVVVVFVLCWLPYHIVLLDLSLCLLSIWRVLPALTALLITL	240
GPCR11	269	SSTVVTPMLNPPIIY	282
00001	241	WLAYVNSCLNPPIIY	254

GPCR11 polypeptides are useful in the generation of antibodies that bind immunospecifically to the GPCR11 polypeptides of the invention. The antibodies are for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the “Anti-GPCR11 Antibodies” section below. The disclosed GPCR11 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR11 epitope is from about amino acids 160 to 170. In another embodiment, a GPCR11 epitope is from about amino acids 210 to 230. In additional embodiments, GPCR11 epitopes are from about amino acids 245 to 260 and from about amino acids 280 to 300. The GPCR11 protein also has value in the development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

GPCR12

Yet another GPCR-like protein of the invention, referred to herein as GPCR12 (alternatively referred to as CG50217-01), is an Olfactory Receptor (“OR”)-like protein.

The novel GPCR12 nucleic acid (SEQ ID NO:96) of 1019 nucleotides encoding a novel Olfactory Receptor-like protein is shown in Table 12A. An open reading frame for the mature protein was identified beginning with a GTG codon which codes for the amino acid Valine at nucleotides 1-3 and ending with a TGA codon at nucleotides 943-45. Putative untranslated regions downstream from the termination codon are underlined in Table 12A, and the start and stop codons are in bold letters.

Table 12A. GPCR12 Nucleotide Sequence (SEQ ID NO:96)

GTGCTGGCTTCAGGGAACAGCTCTTCTCATCCTGTGTCTTCATCCTGCTTGGGAATCCCAGGCCTGGA
GAGTTTCCAGTTGTGGATTGCCTTTCCGTTCTGTGCCACGTATGCTGTGGCTGTTGTTGGAAATATCA
CTCTCCTCCATGTAATCAGAATTGACCACACCCTGCATGAGCCCATGTACCTCTTCTGGCCATGCTG
GCCATCACTGACCTGGTCTCTCTCCTCCTCACTCAACCTAAGATGTTGGCCATATCTGGTTTTCATGC
TCATGAGATTCACTACCATGCCTGCCTCATCCAGGTGTTCTTCATCCATGCCTTTCTTCTGTGGAGT
CTGGGGTGTCTATGGCTATGGCCCTGGACTGCTACGTGGCTATCTGCTTCCCACTCCGACACTCTAGC
ATCCTGACCCCATCGTTCGTGATCAAACCTGGGGACCATCGTGATGCTGAGAGGGCTGCTGTGGGTGAG
CCCCTTCTGCTTCATGGTGTCTAGGATGCCCTTCTGCCAACCAAGCCATTCCCAGTCATACTGTG
AGCACATGGCTGTGCTGAAGTTGGTGTGTGCTGATACAAGCATAAAGTCGTGGGAATGGGCTCTTTGTG
GCCTTCTCTGTGGCTGGCTTTGATATGATTGTGATTGGTATGTCATACGTGATGATTTTGTAGAGCTGT
GCTTCAGTTGCCCTCAGGTGAAGCCCGCCTCAAAGCTTTTAGCACACGTTCTCCCATATCTGTGTCA
TCTTGGCTCTTTATATCCAGCCCTTTTTCTTCTCCTCACCTACCGCTTTGGCCATGATGTGCCCCGA
GTTGTACACATCCTGTTTGCTAATCTCTATCTACTGATACCTCCCATGCTCAACCCCATCATTTATGG

AGTTAGAACCAAACAGATCGGGGACAGGGTTATCCAAGGATGTTGTGGAAACATCCCCCTGAGCAAAGG
GTCAGTGTATCCCCATCACTTACATTGCCCCACTAATGTGGGGACATTAATGAACATTTGACAGGCT

In a search of sequence databases, it was found that the disclosed GPCR12 nucleic acid sequence has 600 of 877 bases (68%) identical to a gb:GENBANK-ID:GGA012570| acc:AJ012570.1 mRNA from Gallus gallus (Gallus gallus DNA sequence downstream of beta-globin locus) ($E = 1.4e^{-73}$).

The disclosed GPCR12 polypeptide (SEQ ID NO: 97) encoded by SEQ ID NO: 96 has 314 amino acid residues using the one-letter code in Table 12B. The SignalP, Psort and/or Hydropathy results predict that GPCR12 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. In alternative embodiments, a GPCR12 polypeptide is located to the Golgi body with a certainty of 0.4600, the endoplasmic reticulum (membrane) with a certainty of 0.3700, or the endoplasmic reticulum (lumen) with a certainty of 0.1000. The most likely cleavage site for a GPCR12 peptide is between amino acids 40 and 41, *i.e.* at the dash in the sequence AVA-VV.

Table 12B. GPCR12 protein sequence (SEQ ID NO:97)

VLASGNSSSHPVSFILLGIPGLESFQLWIAFPFCATYAVAVVGNITLLHVIRIDHTLHEPMYLFLAMLAI
TDLVLSSTQPKMLAIFWFHAHEIQYHACLIQVFFIHAFSSVESGVLMMALDCYVAICFPLRHSSILTP
SVVIKLGITIVMLRGLLVWSPFCFMVSRMPFCQHQAI PQSYCEHMAVLKLVCA DT SISRGNGLFVAFSVAG
FDMIVIGMSYVMILRAVLQLPSGEARLKAFSTRSSHICVILALYIPALFSFLT YRFGHDVPRVVHILFAN
LYLLIPPMLNPIIYGVRTKQIGDRVIQGCCGNIP

Additional SNP variants of GPCR12 are disclosed in Example 3. Expression data for GPCR12 is provided in Example 2.

In a search of a proprietary PatP database, the amino acid sequence of GPCR12 was found to have high homology to other OR-like proteins, as shown in Table 12C.

Table 12C. BLASTX results for GPCR12

	High Score	Smallest Sum Prob P(N)
patp:Sequences producing High-scoring Segment Pairs:		
AAG71726 Human olfactory receptor polypeptide, 314 aa	1607	5.1e-165
AAG71545 Human olfactory receptor polypeptide, 314 aa	924	1.2e-92
AAG72396 Human OR-like polypeptide query sequence, 314 aa	924	1.2e-92
AAG71651 Human olfactory receptor polypeptide, 321 aa	909	4.7e-91
AAE02491 Human CON193 G protein-coupled receptor protein, 321 aa	904	1.6e-90

5	XX:Q9WVD9	EDLHSWIAIPICSMYIVAVICNVLLIFLIVTERSLHEPMPYFFLSMLALAD
	XX:Q9EQQ5	ESVQFWIGIPFCIMYIIALLCNSLLLVVVIKVERSLHEPMPYFLAMLGATD
	XX:Q9H346	EAAHFWIAIPFCAMYLVALVCNAALILVITAMDNALHAPMYLFLCLLSITD
	XX:Q9UKL2	ESVQFWIGIPFCATYLTAMICNSLLLSITKERSLHEPLYIFLGMLGATD
		110 120 130 140 150
10	XX:GCPR12	LVLSSSTOPKMLAIFWFHAEIOYHACLIQVFFIHAFSSVESGVLMMAMAL
	XX:Q9WU90	LVLCSSTILPKMLAIFWLRSHVISYHGCLTQMFFVHAVFATESAVLLAMAF
	XX:Q9WVD9	LLLSTATAPKMLAIFWFHSGISFSGSCVSQMFHIFIFVAESAAILLAMAF
	XX:Q9EQQ5	ISLSTISILPKMLGIFWFHLSITFYDACLQMWLIHTFQGIESGILFAMAM
	XX:Q9H346	LALSSITVPKMLAILWLHAGETSEGGCLAQMFVHSIYALESSILLAMAF
	XX:Q9UKL2	LALASSIMPKMLGIFWFNVPEIYEDSCLLQMWFIHTLQGIESGILVAMAL
15		160 170 180 190 200
20	XX:GCPR12	DCYVAICPLRHSSILTPSVVIKLTIVMLRGLLWSPRCFMVS-RMPFC
	XX:Q9WU90	DRYVAICRPLHYTSILNAVIGKICLACVTRGLLVFEPFVILIE-RLPFC
	XX:Q9WVD9	DRYVAICYPLRYTTILTSSVIGKICTAAVRSFLICFPPIFLVY-RLLYC
	XX:Q9EQQ5	DRYVAICDPLRHASIFTORLLTQICVGVTLRAALFVAPCLFLIKCRLKEY
	XX:Q9H346	DRYVAICNPLRYTTILNHAVIGRICFVGLFRSVAIVSPPIFLLR-RLPYC
	XX:Q9UKL2	DRYVAICYPLRHANITFTHQLVIOICTMVVLRRAAILVAPCLVLIKCRFOFY
25		210 220 230 240 250
	XX:GCPR12	QHQAIPQSYCEHMAVLKLVCA DTSTSRGNGLFVAFSVAGFDMIVIGMSYV
	XX:Q9WU90	GHHIIPHTYCEHMGIAKLACASIKPNTIYGLTVALSVTCMDVVLIATSYI
	XX:Q9WVD9	GKHHIIPHSYCEHMGIA RLACDNITVNIYGLTMALLSTGLDILLIIISYT
	XX:Q9EQQ5	WTTTVVSHSYCEHMAIVKLA AEDVHVNKIYGLFVAFSILGDIIFITLSYI
30	XX:Q9H346	GHRVMTHTYCEHMGIA RLACANITVNIYGLTVALLAMGLDSILIAISYG
	XX:Q9UKL2	HTTVIISHSYCEHMAIVKLA AANVQV NKIYGLFVAFSTVAGFDLTFITLSYI
35		260 270 280 290 300
	XX:GCPR12	MILRAVLQLP SGEARLKAFSTRSSHICVILALYIPALFSFLTYRFGHD-V
	XX:Q9WU90	LILQAVLRRLPSKDAQFRAFSTCGAHICVILVFIYIPAFFSFFTHRFGHH-V
	XX:Q9WVD9	MILRTVFQIPSWAARYKALNTCGSHICVILLFYTPAFFSFFAHRFGGKTV
	XX:Q9EQQ5	RIFITVFKLPQKEARLKAFNTCVAHICVFLFELYLLAFFSFFTHRFGYH-V
	XX:Q9H346	FILHAVFHLPSHDAQHKALSTCGSHIGILLVFIYIPAFFSFLTHRFGHHEV
40	XX:Q9UKL2	QIFITVFRLPQKEAREKAFNTCIAHICVFLQFYLLAFFSFFTHRFGSH-I
45		310 320 330 340 350
	XX:GCPR12	PRVVHILFANLYLLIPPM LNPIIYGVRTKQIGDRVIQGCCGNIP-----
	XX:Q9WU90	PPQVHIILANLYLLVPPV LNPLVYGINTKQIRLRILDFFVKRR-----
	XX:Q9WVD9	PRHIHILVANLYVVPPM LNPIIYGVKTKQIQDRVVFLFSSVSTCQHDSR
	XX:Q9EQQ5	PSYIHILLSNLYLLVPP LNPIVYGVKTKQIRDQVSKILYCNYSY-----
	XX:Q9H346	PKHVHIFLANLYVLVPPV LNPILYGARTKEIRSRLKLLHLGKTSI----
	XX:Q9UKL2	PPYIHILFSSIIYLLVPP LNPLVYCAKTTQIRIHVVVKMFCS-----
50		
55	XX:GCPR12	.
	XX:Q9WU90	-
	XX:Q9WVD9	C
	XX:Q9EQQ5	-
	XX:Q9H346	-
	XX:Q9UKL2	-

stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources. Further expression data for GPCR12 is provided in
5 Example 2.

The nucleic acids and proteins of GPCR12 are useful in potential diagnostic and therapeutic applications implicated in various GPCR-related pathological diseases and/or disorders, and/or in various other pathologies, as described above.

The polypeptides can be used as immunogens to produce antibodies specific for the
10 invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the OR -like protein may be useful in gene therapy, and the OR-like protein may be useful when administered to a subject in need thereof. The novel nucleic acid encoding OR-like protein, and the OR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the
15 presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods and other diseases, disorders and conditions of the like.

These antibodies may be generated according to methods known in the art, using
20 predictions from hydrophobicity charts, as described in the "Anti-GPCR12 Antibodies" section below. The disclosed GPCR12 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR12 epitope is from about amino acids 80 to 95. In another embodiment, a GPCR12 epitope is from about amino acids 170 to 185. In further embodiments, a GPCR12 epitope is from about 230 to 247. In yet
25 further specific embodiments, a GPCR12 epitope is from about amino acids 290 to 325.

GPCR12 Nucleic Acids and Polypeptides

A summary of the GPCR12 nucleic acids and proteins of the invention is provided in Table 13.

TABLE 13: Summary Of Nucleic Acids And Proteins Of The Invention

GPCRX Name	Tables	Clone; Description of Homolog	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO
GPCR1a	1A, 1B	OR-like; GPCR-like	1	2
GPCR1b	1C, 1D	OR-like; GPCR-like	3	4
GPCR1c	1E, 1F	OR-like; GPCR-like	5	6
GPCR1d	1G, 1H	OR-like; GPCR-like	7	8
GPCR1e	1I, 1J	OR-like; GPCR-like	9	10
GPCR1f	1L, 1L	OR-like; GPCR-like	11	12
GPCR2	2A, 2B	OR-like; GPCR-like	20	21
GPCR3	3A, 3B	OR-like; GPCR-like	27	28
GPCR4a	4A, 4B	OR-like; GPCR-like	34	35
GPCR4b	4C, 4D	OR-like; GPCR-like	36	37
GPCR5	5A, 5B	OR-like; GPCR-like	43	44
GPCR6	6A, 6B	OR-like; GPCR-like	50	51
GPCR7a	7A, 7B	OR-like; GPCR-like	57	58
GPCR7b	7C, 7D	OR-like; GPCR-like	59	60
GPCR8	8A, 8B	OR-like; GPCR-like	66	67
GPCR9	9A, 9B	OR-like; GPCR-like	73	74
GPCR10a	10A, 10B	OR-like; GPCR-like	80	81
GPCR10b	10C, 10D	OR-like; GPCR-like	82	83
GPCR11	11A, 11B	OR-like; GPCR-like	89	90
GPCR12	12A, 12B	OR-like; GPCR-like	96	97

One aspect of the invention pertains to isolated nucleic acid molecules that encode GPCRX polypeptides or biologically active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify GPCRX-encoding nucleic acids (*e.g.*, GPCRX mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of GPCRX nucleic acid molecules. As used herein, the term “nucleic acid molecule” is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An GPCRX nucleic acid can encode a mature GPCRX polypeptide. As used herein, a “mature” form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring

polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full-length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an ORF described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an ORF, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term "probes", as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one, which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated GPCR_X nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb,

1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 as a hybridization probe, GPCR_X molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to GPCR_X nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20,

27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, or a complement thereof.

Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID

5 NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, or a portion of this nucleotide sequence (*e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an GPCR_X polypeptide). A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 is one that is sufficiently complementary to
10 the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, thereby forming a stable duplex.

As used herein, the term “complementary” refers to Watson-Crick or Hoogsteen base
15 pairing between nucleotides units of a nucleic acid molecule, and the term “binding” means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or
20 compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific
25 hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs
30 are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a

similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or

5 analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a
10 computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or
15 variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of GPCR_X polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous
20 nucleotide sequences include nucleotide sequences encoding for an GPCR_X polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence
25 does not, however, include the exact nucleotide sequence encoding human GPCR_X protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 or 97, as well as a polypeptide possessing GPCR_X biological activity. Various biological activities of the GPCR_X proteins are described below.

30 As used herein, "identical" residues correspond to those residues in a comparison between two sequences where the equivalent nucleotide base or amino acid residue in an alignment of two sequences is the same residue. Residues are alternatively described as

“similar” or “positive” when the comparisons between two sequences in an alignment show that residues in an equivalent position in a comparison are either the same amino acid or a conserved amino acid as defined below.

An GPCR_X polypeptide is encoded by the open reading frame (“ORF”) of an GPCR_X nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG “start” codon and terminates with one of the three “stop” codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, e.g., a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human GPCR_X genes allows for the generation of probes and primers designed for use in identifying and/or cloning GPCR_X homologues in other cell types, e.g. from other tissues, as well as GPCR_X homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96.

Probes based on the human GPCR_X nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an GPCR_X protein, such as by measuring a level of an GPCR_X-encoding nucleic acid in a sample of cells from a subject e.g., detecting GPCR_X mRNA levels or determining whether a genomic GPCR_X gene has been mutated or deleted.

“A polypeptide having a biologically-active portion of an GPCR_X polypeptide” refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of GPCR_X" can be prepared by isolating a portion SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 that encodes a polypeptide having an GPCR_X biological activity (the biological activities of the GPCR_X proteins are described below), expressing the encoded portion of GPCR_X protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of GPCR_X.

GPCR_X Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 due to degeneracy of the genetic code and thus encode the same GPCR_X proteins as that encoded by the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97.

In addition to the human GPCR_X nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the GPCR_X polypeptides may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the GPCR_X genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an GPCR_X protein, preferably a vertebrate GPCR_X protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the GPCR_X genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the GPCR_X polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the GPCR_X polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding GPCR_X proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the GPCR_X cDNAs of the invention can be isolated based on their

homology to the human GPCR_X nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding GPCR_X proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5 °C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at T_m, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in

which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization

conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may
5 be used are well known in the art (e.g., as employed for cross-species hybridizations). See, e.g., Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* 78: 6789-6792.

10 **Conservative Mutations**

In addition to naturally-occurring allelic variants of GPCR_X sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 thereby leading to changes in the amino acid sequences of the
15 encoded GPCR_X proteins, without altering the functional ability of said GPCR_X proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the GPCR_X proteins without altering their
20 biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the GPCR_X proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding GPCR_X
25 proteins that contain changes in amino acid residues that are not essential for activity. Such GPCR_X proteins differ in amino acid sequence from SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous
30 to the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60,

67, 74, 81, 83, 90 and 97; more preferably at least about 70% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97; still more preferably at least about 80% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97; even more preferably at least about 90% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97; and most preferably at least about 95% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97.

An isolated nucleic acid molecule encoding an GPCR_X protein homologous to the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the GPCR_X protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an GPCR_X coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for GPCR_X biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, the encoded protein can be expressed

by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant GPCR_X protein can be assayed for (i) the ability to form protein:protein interactions with other GPCR_X proteins, other cell-surface proteins, or biologically-active portions thereof, (ii) complex formation between a mutant GPCR_X protein and an GPCR_X ligand; or (iii) the ability of a mutant GPCR_X protein to bind to an intracellular target protein or biologically-active portion thereof; (e.g. avidin proteins).

In yet another embodiment, a mutant GPCR_X protein can be assayed for the ability to regulate a specific biological function (e.g., regulation of insulin release).

Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire GPCR_X coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an GPCR_X protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97, or antisense nucleic acids complementary to an GPCR_X nucleic acid sequence of SEQ ID

NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an GPCR_X protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the GPCR_X protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the GPCR_X protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of GPCR_X mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of GPCR_X mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of GPCR_X mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil,

2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an GPCR_X protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (*e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other. See, *e.g.*, Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, *e.g.*, Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a chimeric RNA-DNA analogue (see, *e.g.*, Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330).

Ribozymes and PNA Moieties

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave GPCR_X mRNA transcripts to thereby inhibit translation of GPCR_X mRNA. A ribozyme having specificity for an GPCR_X-encoding nucleic acid can be designed based upon the nucleotide sequence of an GPCR_X cDNA disclosed herein (*i.e.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an GPCR_X-encoding mRNA. See, *e.g.*, U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* GPCR_X mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, GPCR_X gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the GPCR_X nucleic acid (*e.g.*, the GPCR_X promoter and/or enhancers) to form triple helical structures that prevent transcription of the GPCR_X gene in target cells. See, *e.g.*, Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the GPCR_X nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. See, *e.g.*, Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (*e.g.*, DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under

conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

PNAs of GPCR_X can be used in therapeutic and diagnostic applications. For example, PNA_s can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNA_s of GPCR_X can also be used, for example, in the analysis of single base pair mutations in a gene (*e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S₁ nucleases (*see*, Hyrup, *et al.*, 1996. *supra*); or as probes or primers for DNA sequence and hybridization (*see*, Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNA_s of GPCR_X can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of GPCR_X can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.*, RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see*, Hyrup, *et al.*, 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.*, 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.*, Mag, *et al.*, 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.*, Finn, *et al.*, 1996. *supra*. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.*, Petersen, *et al.*, 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across

the cell membrane (*see, e.g., Letsinger, et al., 1989. Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; Lemaitre, *et al., 1987. Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g., PCT Publication No. WO 89/10134*). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g., Krol, et al., 1988. BioTechniques* 6:958-976) or intercalating agents (*see, e.g., Zon, 1988. Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.,* a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

GPCRX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of GPCR_X polypeptides whose sequences are provided in SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97 while still encoding a protein that maintains its GPCR_X activities and physiological functions, or a functional fragment thereof.

In general, an GPCR_X variant that preserves GPCR_X-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated GPCR_X proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-GPCR_X antibodies. In one embodiment, native GPCR_X proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, GPCR_X proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an GPCR_X protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the GPCR_X protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of GPCR_X proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes preparations of GPCR_X proteins having less than about 30% (by dry weight) of non-GPCR_X proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-GPCR_X proteins, still more preferably less than about 10% of non-GPCR_X proteins, and most preferably less than about 5% of non-GPCR_X proteins. When the GPCR_X protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the GPCR_X protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of GPCR_X proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of GPCR_X proteins having less than about 30% (by dry weight) of chemical precursors or non-GPCR_X chemicals, more preferably less than about 20% chemical precursors or non-GPCR_X chemicals, still more preferably less than about 10% chemical precursors or non-GPCR_X chemicals, and most preferably less than about 5% chemical precursors or non-GPCR_X chemicals.

Biologically-active portions of GPCR_X proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the GPCR_X proteins (*e.g.*, the amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97) that include fewer amino acids than the full-length GPCR_X proteins, and exhibit at least one activity of an GPCR_X protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the GPCR_X protein. A biologically-active portion of an GPCR_X protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native GPCR_X protein.

In an embodiment, the GPCR_X protein has an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97. In other embodiments, the GPCR_X protein is substantially homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97, and retains the functional activity of the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the GPCR_X protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97, and retains the functional activity of the GPCR_X proteins of SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97.

Determining Homology Between Two or More Sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See*, Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or

99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

Chimeric and Fusion Proteins

The invention also provides GPCR_X chimeric or fusion proteins. As used herein, an GPCR_X "chimeric protein" or "fusion protein" comprises an GPCR_X polypeptide operatively-linked to a non-GPCR_X polypeptide. An "GPCR_X polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an GPCR_X protein (SEQ ID NOS:2, 4, 6, 8, 10, 12, 21, 28, 35, 37, 44, 51, 58, 60, 67, 74, 81, 83, 90 and 97), whereas a "non-GPCR_X polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the GPCR_X protein, *e.g.*, a protein that is different from the GPCR_X protein and that is derived from the same or a different organism. Within an GPCR_X fusion protein the GPCR_X polypeptide can correspond to all or a portion of an GPCR_X protein. In one embodiment, an GPCR_X fusion protein comprises at least one biologically-active portion of an GPCR_X protein. In another embodiment, an GPCR_X fusion protein comprises at least two biologically-active portions of an GPCR_X protein. In yet another embodiment, an GPCR_X fusion protein comprises at least three biologically-active portions of an GPCR_X protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the GPCR_X polypeptide and the non-GPCR_X polypeptide are fused

in-frame with one another. The non-GPCR_X polypeptide can be fused to the N-terminus or C-terminus of the GPCR_X polypeptide.

In one embodiment, the fusion protein is a GST-GPCR_X fusion protein in which the GPCR_X sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant GPCR_X polypeptides.

In another embodiment, the fusion protein is an GPCR_X protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of GPCR_X can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an GPCR_X-immunoglobulin fusion protein in which the GPCR_X sequences are fused to sequences derived from a member of the immunoglobulin protein family. The GPCR_X-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an GPCR_X ligand and an GPCR_X protein on the surface of a cell, to thereby suppress GPCR_X-mediated signal transduction *in vivo*. The GPCR_X-immunoglobulin fusion proteins can be used to affect the bioavailability of an GPCR_X cognate ligand. Inhibition of the GPCR_X ligand/GPCR_X interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the GPCR_X-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-GPCR_X antibodies in a subject, to purify GPCR_X ligands, and in screening assays to identify molecules that inhibit the interaction of GPCR_X with an GPCR_X ligand.

An GPCR_X chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two

consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.,* Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.,* a GST polypeptide). An

- 5 GPCR_X-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the GPCR_X protein.

GPCR_X Agonists and Antagonists

- The invention also pertains to variants of the GPCR_X proteins that function as either GPCR_X agonists (*i.e.,* mimetics) or as GPCR_X antagonists. Variants of the GPCR_X protein
10 can be generated by mutagenesis (*e.g.,* discrete point mutation or truncation of the GPCR_X protein). An agonist of the GPCR_X protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the GPCR_X protein. An antagonist of the GPCR_X protein can inhibit one or more of the activities of the naturally occurring form of the GPCR_X protein by, for example, competitively binding to a downstream or upstream
15 member of a cellular signaling cascade which includes the GPCR_X protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the GPCR_X proteins.

- 20 Variants of the GPCR_X proteins that function as either GPCR_X agonists (*i.e.,* mimetics) or as GPCR_X antagonists can be identified by screening combinatorial libraries of mutants (*e.g.,* truncation mutants) of the GPCR_X proteins for GPCR_X protein agonist or antagonist activity. In one embodiment, a variegated library of GPCR_X variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene
25 library. A variegated library of GPCR_X variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential GPCR_X sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.,* for phage display) containing the set of GPCR_X sequences therein. There are a variety of methods which can be used to produce
30 libraries of potential GPCR_X variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate

set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential GPCR_X sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. See, e.g., Narang, 1983. *Tetrahedron* 39: 3; Itakura, et al., 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, et al., 1984. *Science* 198: 1056; Ike, et al., 1983. *Nucl. Acids Res.* 11: 477.

Polypeptide Libraries

In addition, libraries of fragments of the GPCR_X protein coding sequences can be used to generate a variegated population of GPCR_X fragments for screening and subsequent selection of variants of an GPCR_X protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an GPCR_X coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S₁ nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the GPCR_X proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of GPCR_X proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify GPCR_X variants. See, e.g., Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, et al., 1993. *Protein Engineering* 6:327-331.

Anti-GPCRX Antibodies

Also included in the invention are antibodies to GPCR_X proteins, or fragments of GPCR_X proteins. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, F_{ab}' and F_{(ab')₂} fragments, and an F_{ab} expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated GPCR_X-related protein of the invention may be intended to serve as an antigen, or a portion or fragment thereof, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of GPCR_X-related protein that is located on the surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human GPCR_X-related protein sequence will indicate which regions of a GPCR_X-related protein are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art,

including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, e.g., Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each of which is incorporated herein by reference in its entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, *Antibodies: A Laboratory Manual*, Harlow and Lane, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

Polyclonal Antibodies

For the production of polyclonal antibodies, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory agents. Additional examples of adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

10 **Monoclonal Antibodies**

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE*, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused,

immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

5 Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human
10 myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., MONOCLONAL ANTIBODY PRODUCTION TECHNIQUES AND APPLICATIONS, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for
15 the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by
20 the Scatchard analysis of Munson and Pollard, *Anal. Biochem.*, 107:220 (1980). Preferably, antibodies having a high degree of specificity and a high binding affinity for the target antigen are isolated.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this
25 purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel
30 electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of

the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, *Nature* 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

Humanized Antibodies

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are

those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)).

5 Human Antibodies

Fully human antibodies relate to antibody molecules in which essentially the entire sequences of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell
10 hybridoma technique (see Kozbor, et al., 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or
15 by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc., pp. 77-96).

In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)). Similarly, human antibodies can be made by
20 introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825;
25 5,625,126; 5,633,425; 5,661,016, and in Marks et al. (*Bio/Technology* 10, 779-783 (1992)); Lonberg et al. (*Nature* 368 856-859 (1994)); Morrison (*Nature* 368, 812-13 (1994)); Fishwild et al, (*Nature Biotechnology* 14, 845-51 (1996)); Neuberger (*Nature Biotechnology* 14, 826 (1996)); and Lonberg and Huszar (*Intern. Rev. Immunol.* 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals
30 which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in

the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the XenomouseTM as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that

binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

F_{ab} Fragments and Single Chain Antibodies

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (see e.g., U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see e.g., Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotype to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an F_{(ab)2} fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an F_{(ab)2} fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, 1991 *EMBO J.*, 10:3655-3659.

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant

region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')₂ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to

cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., *J. Immunol.* 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (Fc γ R), such as Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

Effector Function Engineering

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989).

Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin,

Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples
5 include ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimide HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as
10 glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-
15 methyl-diethylene triamine-pentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation
20 using a clearing agent and then administration of a "ligand" (e.g., avidin) that is in turn conjugated to a cytotoxic agent.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and
25 other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an GPCR protein is facilitated by generation of hybridomas that bind to the fragment of an GPCR protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an GPCR protein, or derivatives, fragments, analogs or homologs thereof, are also provided
30 herein.

Anti-GPCR antibodies may be used in methods known within the art relating to the localization and/or quantitation of an GPCR protein (e.g., for use in measuring levels of the

GPCRX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for GPCR_X proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-GPCR_X antibody (*e.g.*, monoclonal antibody) can be used to isolate an GPCR_X polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-GPCR_X antibody can facilitate the purification of natural GPCR_X polypeptide from cells and of recombinantly-produced GPCR_X polypeptide expressed in host cells. Moreover, an anti-GPCR_X antibody can be used to detect GPCR_X protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the GPCR_X protein. Anti-GPCR_X antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

GPCR_X Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an GPCR_X protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous

replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, GPCR proteins, mutant forms of GPCR proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of GPCR_X proteins in prokaryotic or eukaryotic cells. For example, GPCR_X proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel,
5 GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion
10 or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression
15 vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL
20 (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION
25 TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY
30 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.*,

Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the GPCR_X expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, GPCR_X can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European

Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the α -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to GPCR_X mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, GPCR_X protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium

chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding GPCR_X or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) GPCR_X protein. Accordingly, the invention further provides methods for producing GPCR_X protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding GPCR_X protein has been introduced) in a suitable medium such that GPCR_X protein is produced. In another embodiment, the method further comprises isolating GPCR_X protein from the medium or the host cell.

Transgenic GPCR_X Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which GPCR_X protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous GPCR_X sequences have been introduced into their genome or homologous recombinant animals in which endogenous GPCR_X sequences have been altered. Such animals are useful for studying the function and/or activity of GPCR_X protein and for identifying and/or evaluating modulators of GPCR_X protein activity. As used herein, a "transgenic animal" is a

non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous GPCR_X gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing GPCR_X-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human GPCR_X cDNA sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human GPCR_X gene, such as a mouse GPCR_X gene, can be isolated based on hybridization to the human GPCR_X cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the GPCR_X transgene to direct expression of GPCR_X protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the GPCR_X transgene in its genome and/or expression of GPCR_X mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding GPCR_X protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an GPCR_X gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the GPCR_X gene. The GPCR_X gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96), but more preferably, is a non-human homologue of a human GPCR_X gene. For example, a mouse homologue of human GPCR_X gene of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 can be used to construct a homologous recombination vector suitable for altering an endogenous GPCR_X gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous GPCR_X gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous GPCR_X gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous GPCR_X protein). In the homologous recombination vector, the altered portion of the GPCR_X gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the GPCR_X gene to allow for homologous recombination to occur between the exogenous GPCR_X gene carried by the vector and an endogenous GPCR_X gene in an embryonic stem cell. The additional flanking GPCR_X nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.*, Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is then introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced GPCR_X gene has homologously-recombined with the endogenous GPCR_X gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for

constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, See, e.g., Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*. See, O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (e.g., a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (e.g., the somatic cell) is isolated.

Pharmaceutical Compositions

The GPCR_X nucleic acid molecules, GPCR_X proteins, and anti-GPCR_X antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like,

compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof.

The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an GPCR_X protein or anti-GPCR_X antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be

achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express GPCR_X protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect GPCR_X mRNA (*e.g.*, in a biological sample) or a genetic lesion in an GPCR_X gene, and to modulate GPCR_X activity, as described further, below. In addition, the GPCR_X proteins can be used to screen drugs or compounds that modulate the GPCR_X protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of GPCR_X protein or production of GPCR_X protein forms that have decreased or aberrant activity compared to GPCR_X wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease (possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-GPCR_X antibodies of the invention can be used to detect and isolate GPCR_X proteins and modulate GPCR_X activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to GPCR_X proteins or have a stimulatory or inhibitory effect on, *e.g.*, GPCR_X protein expression or GPCR_X protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an GPCR_X protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner,

U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

5 In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of GPCR_X protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an GPCR_X protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the GPCR_X protein can be
10 accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the GPCR_X protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ¹²⁵I, ³⁵S, ¹⁴C, or ³H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting.
15 Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of GPCR_X protein, or a biologically-active portion thereof, on the cell surface with a known compound
20 which binds GPCR_X to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR_X protein, wherein determining the ability of the test compound to interact with an GPCR_X protein comprises determining the ability of the test compound to preferentially bind to GPCR_X protein or a biologically-active portion thereof as compared to the known compound.

25 In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of GPCR_X protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the GPCR_X protein or biologically-active portion thereof. Determining the ability of the test compound to modulate
30 the activity of GPCR_X or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the GPCR_X protein to bind to or interact with an GPCR_X target molecule. As used herein, a "target molecule" is a molecule with which an

GPCRX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an GPCR_X interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An GPCR_X target molecule can be a non-GPCR_X molecule or an GPCR_X protein or polypeptide of the invention. In one embodiment, an GPCR_X target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound GPCR_X molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with GPCR_X.

Determining the ability of the GPCR_X protein to bind to or interact with an GPCR_X target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the GPCR_X protein to bind to or interact with an GPCR_X target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca²⁺, diacylglycerol, IP₃, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an GPCR_X-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an GPCR_X protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the GPCR_X protein or biologically-active portion thereof. Binding of the test compound to the GPCR_X protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the GPCR_X protein or biologically-active portion thereof with a known compound which binds GPCR_X to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR_X protein, wherein determining the ability of the test compound to interact with an GPCR_X protein comprises determining the ability of the test compound to preferentially bind to GPCR_X or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting GPCR_X protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the GPCR_X protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCR_X can be accomplished, for example, by determining the ability of the GPCR_X protein to bind to an GPCR_X target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of GPCR_X protein can be accomplished by determining the ability of the GPCR_X protein further modulate an GPCR_X target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the GPCR_X protein or biologically-active portion thereof with a known compound which binds GPCR_X protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR_X protein, wherein determining the ability of the test compound to interact with an GPCR_X protein comprises determining the ability of the GPCR_X protein to preferentially bind to or modulate the activity of an GPCR_X target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of GPCR_X protein. In the case of cell-free assays comprising the membrane-bound form of GPCR_X protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of GPCR_X protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either GPCR_X protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to GPCR_X protein, or interaction of

GPCRX protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-GPCR_X fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or GPCR_X protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of GPCR_X protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the GPCR_X protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated GPCR_X protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with GPCR_X protein or target molecules, but which do not interfere with binding of the GPCR_X protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or GPCR_X protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the GPCR_X protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the GPCR_X protein or target molecule.

In another embodiment, modulators of GPCR_X protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of GPCR_X mRNA or protein in the cell is determined. The level of expression of GPCR_X mRNA or protein in the presence of the candidate compound is compared to the level of expression of

GPCRX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of GPCRX mRNA or protein expression based upon this comparison. For example, when expression of GPCRX mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of GPCRX mRNA or protein expression. Alternatively, when expression of GPCRX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of GPCRX mRNA or protein expression. The level of GPCRX mRNA or protein expression in the cells can be determined by methods described herein for detecting GPCRX mRNA or protein.

In yet another aspect of the invention, the GPCRX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with GPCRX ("GPCRX-binding proteins" or "GPCRX-bp") and modulate GPCRX activity. Such GPCRX-binding proteins are also likely to be involved in the propagation of signals by the GPCRX proteins as, for example, upstream or downstream elements of the GPCRX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for GPCRX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an GPCRX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with GPCRX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the GPCR_X sequences, SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, or fragments or derivatives thereof, can be used to map the location of the GPCR_X genes, respectively, on a chromosome. The mapping of the GPCR_X sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, GPCR_X genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the GPCR_X sequences. Computer analysis of the GPCR_X sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the GPCR_X sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be

established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. *See, e.g., D'Eustachio, et al., 1983. Science 220: 919-924.* Somatic cell hybrids containing only fragments of human
5 chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the GPCR sequences to design oligonucleotide primers, sub-
10 localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes
15 can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000
20 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see, Verma, et al., HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for
25 marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical
30 position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g., in McKusick, MENDELIAN INHERITANCE IN MAN*, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes

and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the GPCR_X gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

The GPCR_X sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the GPCR_X sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The GPCR_X sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single

nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining GPCR_X protein and/or nucleic acid expression as well as GPCR_X activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant GPCR_X expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with GPCR_X protein, nucleic acid expression or activity. For example, mutations in an GPCR_X gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with GPCR_X protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining GPCR_X protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or

prophylactic agents for that individual (referred to herein as "pharmacogenomics").

Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCR_X in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of GPCR_X in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting GPCR_X protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes GPCR_X protein such that the presence of GPCR_X is detected in the biological sample. An agent for detecting GPCR_X mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to GPCR_X mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length GPCR_X nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 11, 20, 27, 34, 36, 43, 50, 57, 59, 66, 73, 80, 82, 89 and 96, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to GPCR_X mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting GPCR_X protein is an antibody capable of binding to GPCR_X protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a

subject. That is, the detection method of the invention can be used to detect GPCR_X mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of GPCR_X mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of GPCR_X protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of GPCR_X genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of GPCR_X protein include introducing into a subject a labeled anti-GPCR_X antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting GPCR_X protein, mRNA, or genomic DNA, such that the presence of GPCR_X protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of GPCR_X protein, mRNA or genomic DNA in the control sample with the presence of GPCR_X protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of GPCR_X in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting GPCR_X protein or mRNA in a biological sample; means for determining the amount of GPCR_X in the sample; and means for comparing the amount of GPCR_X in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect GPCR_X protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant GPCR_X expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with GPCR_X protein, nucleic acid expression or activity.

Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant GPCR_X expression or activity in which a test sample is obtained from a subject and GPCR_X protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of GPCR_X protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant GPCR_X expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant GPCR_X expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant GPCR_X expression or activity in which a test sample is obtained and GPCR_X protein or nucleic acid is detected (*e.g.*, wherein the presence of GPCR_X protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant GPCR_X expression or activity).

The methods of the invention can also be used to detect genetic lesions in an GPCR_X gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an GPCR_X-protein, or the misexpression of the GPCR_X gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (i) a deletion of one or more nucleotides from an GPCR_X gene; (ii) an addition of one or more nucleotides to an GPCR_X gene; (iii) a substitution of one or more nucleotides of an GPCR_X gene, (iv) a chromosomal rearrangement of an GPCR_X gene; (v) an alteration in the level of a messenger RNA transcript of an GPCR_X gene, (vi) aberrant modification of an GPCR_X gene, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild-type splicing

pattern of a messenger RNA transcript of an GPCR_X gene, (viii) a non-wild-type level of an GPCR_X protein, (ix) allelic loss of an GPCR_X gene, and (x) inappropriate post-translational modification of an GPCR_X protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an GPCR_X gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the GPCR_X-gene (*see*, Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an GPCR_X gene under conditions such that hybridization and amplification of the GPCR_X gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see*, Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see*, Kwok, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Q β Replicase (*see*, Lizardi, *et al.*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an GPCR_X gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared.

Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g., U.S. Patent No. 5,493,531*) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

5 In other embodiments, genetic mutations in GPCR_X can be identified by hybridizing a sample and control nucleic acids, *e.g.,* DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotide probes. *See, e.g., Cronin, et al., 1996. Human Mutation* 7: 244-255; Kozal, *et al., 1996. Nat. Med.* 2: 753-759. For example, genetic mutations in GPCR_X can be identified in two dimensional arrays containing light-generated
10 DNA probes as described in Cronin, *et al., supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller,
15 specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the GPCR_X gene and detect mutations by comparing the
20 sequence of the sample GPCR_X with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g., Naeve, et al., 1995. Biotechniques* 19: 448), including sequencing by mass spectrometry (*see, e.g., PCT International Publication No. WO 94/16101; Cohen, et al., 1996. Adv. Chromatography* 36: 127-162; and Griffin, *et al., 1993. Appl. Biochem. Biotechnol.* 38: 147-159).

25 Other methods for detecting mutations in the GPCR_X gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or
30 RNA/DNA heteroduplexes. *See, e.g., Myers, et al., 1985. Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type GPCR_X sequence with

potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S₁ nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, e.g., Cotton, *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.*, 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in GPCR_X cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. See, e.g., Hsu, *et al.*, 1994. *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an GPCR_X sequence, e.g., a wild-type GPCR_X sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, e.g., U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in GPCR_X genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. See, e.g., Orita, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA*: 86: 2766; Cotton, 1993. *Mutat. Res.* 285: 125-144; Hayashi, 1992. *Genet. Anal. Tech. Appl.* 9: 73-79. Single-stranded DNA fragments of sample and control GPCR_X nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one

embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g., Keen, et al., 1991. Trends Genet. 7: 5.*

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g., Myers, et al., 1985. Nature 313: 495.* When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g., Rosenbaum and Reissner, 1987. Biophys. Chem. 265: 12753.*

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g., Saiki, et al., 1986. Nature 324: 163; Saiki, et al., 1989. Proc. Natl. Acad. Sci. USA 86: 6230.* Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g., Gibbs, et al., 1989. Nucl. Acids Res. 17: 2437-2448*) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g., Prossner, 1993. Tibtech. 11: 238*). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g., Gasparini, et al., 1992. Mol. Cell Probes 6: 1.* It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g., Barany, 1991. Proc. Natl. Acad. Sci. USA 88: 189.* In such cases, ligation will occur only if there is a

perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an GPCR_X gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which GPCR_X is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on GPCR_X activity (*e.g.*, GPCR_X gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of GPCR_X protein, expression of GPCR_X nucleic acid, or mutation content of GPCR_X genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of GPCR_X protein, expression of GPCR_X nucleic acid, or mutation content of GPCR_X genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness

phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an GPCR_X modulator, such as a modulator identified by one of the exemplary screening assays described herein.

5 **Monitoring of Effects During Clinical Trials**

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCR_X (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase GPCR_X gene expression, protein levels, or upregulate GPCR_X activity, can be monitored in clinical trials of subjects exhibiting decreased GPCR_X gene expression, protein levels, or downregulated GPCR_X activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease GPCR_X gene expression, protein levels, or downregulate GPCR_X activity, can be monitored in clinical trials of subjects exhibiting increased GPCR_X gene expression, protein levels, or upregulated GPCR_X activity. In such clinical trials, the expression or activity of GPCR_X and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including GPCR_X, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates GPCR_X activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of GPCR_X and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of GPCR_X or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide,

peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an GPCR_X protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the GPCR_X protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the GPCR_X protein, mRNA, or genomic DNA in the pre-administration sample with the GPCR_X protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of GPCR_X to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of GPCR_X to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant GPCR_X expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Osteodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below.

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize

activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant GPCR_X expression or activity, by administering to the subject an agent that modulates GPCR_X expression or at least one GPCR_X activity. Subjects at risk for a disease that is caused or contributed to by aberrant GPCR_X expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation

of symptoms characteristic of the GPCR_X aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of GPCR_X aberrancy, for example, an GPCR_X agonist or GPCR_X antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating GPCR_X expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of GPCR_X protein activity associated with the cell. An agent that modulates GPCR_X protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an GPCR_X protein, a peptide, an GPCR_X peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more GPCR_X protein activity. Examples of such stimulatory agents include active GPCR_X protein and a nucleic acid molecule encoding GPCR_X that has been introduced into the cell. In another embodiment, the agent inhibits one or more GPCR_X protein activity. Examples of such inhibitory agents include antisense GPCR_X nucleic acid molecules and anti-GPCR_X antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an GPCR_X protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) GPCR_X expression or activity. In another embodiment, the method involves administering an GPCR_X protein or nucleic acid molecule as therapy to compensate for reduced or aberrant GPCR_X expression or activity.

Stimulation of GPCR_X activity is desirable in situations in which GPCR_X is abnormally downregulated and/or in which increased GPCR_X activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (e.g., cancer or immune

associated disorders). Another example of such a situation is where the subject has a gestational disease (e.g., preclampsia).

Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Prophylactic and Therapeutic Uses of the Compositions of the Invention

The GPCR_X nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the GPCR_X protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the GPCR_X protein, and the GPCR_X protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the

presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

EXAMPLES

Example 1. Identification of GPCR_X clones

All novel GPCR_X target sequences identified in the present invention were subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. Table 14A shows the sequences of the PCR primers used for obtaining different clones. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such primers were designed based on in silico predictions for the full length cDNA, part (one or more exons) of the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related human sequences from other species. These primers were then employed in PCR amplification based on the following pool of human cDNAs: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain - hippocampus, brain - substantia nigra, brain - thalamus, brain - whole, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. Usually the resulting amplicons were gel purified, cloned and sequenced to high redundancy. The PCR product derived from exon linking was cloned into the pCR2.1 vector from Invitrogen. The resulting bacterial clone has an insert covering the entire open reading frame cloned into the pCR2.1 vector. Table 14B shows a list of these bacterial clones. The resulting sequences from all clones were assembled with themselves, with other fragments in CuraGen Corporation's database and with public ESTs. Fragments and ESTs were included as components for an assembly when the extent of their identity with another component of the assembly was at

least 95% over 50 bp. In addition, sequence traces were evaluated manually and edited for corrections if appropriate. These procedures provide the sequence reported herein.

Table 14A. PCR Primers for Exon Linking

GPCR Clone	Primer 1 (5' to 3')	SEQ ID NO	Primer 2 (5' to 3')	SEQ ID NO
GPCR1a- GPCR1e	CCCCATACTGTGGATCATGGCAAAT	103	GGCTCATCATTGTGCCTTGCAAAG	104
GPCR1f	as above	103	CTCATCATTGTGCCTTGCAAAGGC	105
GPCR2	GACTAAATGATGGACAACCACTCTAGT	106	AGCTAATCTTTCAGGAGTTGACAGC	107
GPCR3	CAATGATGGAAATAGCCAATGTGAG	108	GAGTCTCTAAATTTGCGCCAGCTT	109
GPCR4a	AGCTGTGGACCATCTCTTCAGAACTCT	110	CTCACCTGGAGGCCCGACTC	111
GPCR4b	TGCTCTTCCCTCTGTGCTCAGC	112	GATGGCCTCAGCTACTAACCTGAGAC	113
GPCR6	GTCAGCCTCCAATATCACCTTAACA	114	CCTCTACATATCCTTTCTTGGGAATAC	115
GPCR7a	CCATGGAGGCTGCCAATGAGTCTT-	116	AGTTGCCAGTGTGGGTGATGCAGT	117
GPCR7b	ATGGGTGAACCACTCCTACACAGATG	118	GTTCAGTGCTGGCTGCCAATC	119
GPCR8	TCTCTGTTTCTCAGGGATTGAGAAAG	120	TCTACACTCGGGCAACCACAATT	121
GPCR9	ATTATGGAAACACAGAACCTCACAGTG	122	TCTCCGTTCTTGCTTTTCTCTTTCTTC	123
GPCR10	ATGGCTGGATCTCTATTCCTTCTGCT	124	CTGTGGGCTTTATGTCCAAAACCTCCT	125

Table 14B. Physical Clones for PCR products

GPCR Clone	Bacterial Clone
GPCR1a- GPCR1d	111973::AC073079.698284.L10
GPCR1e	111973::AC073079.698284.L14
GPCR1f	111965::AC073079.698320.D13
GPCR3	78355::sggc draft ba656o22 20000731. 698081.G1 FLC ELT
GPCR4a	93200::sggc draft ba112j3 20000804.698247.N5
GPCR4b	93327::sggc draft ba112j3 20000804.698200.B13
GPCR6	118118::AC010930.698337.M3 and 118118::AC010930.698337.M5
GPCR7	119262::ba163b6 A.698349.I5
GPCR8	116713::GMAC011904 A.698344.A11; 116713::GMAC011904 A.698344.A23
GPCR9	115574::GMAL163152 D.698322.B3; 115574::GMAL163152 D.698322.B21
GPCR11	116796::GMAC002555 A.698344.G1; 116796::GMAC002555 A.698344.G5
GPCR12	114812::AC011711.698329.F12; 114820::AC026331.698329.J4

The protein [PF00001 7tm_1] OLFACTORY RECEPTOR 15 (OR3) - Mus musculus (Mouse), 312 amino acids, was used in a TBLASTN search against human genomic sequences. The genomic clone AC073079 was identified as containing a full length gene similar to this olfactory receptor. Primers were designed based on in silico these predictions for the full length or part (one or more exons) of the DNA/protein sequence of the invention.

Example 2. Quantitative expression analysis of clones in various cells and tissues

The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR). RTQ PCR was performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing normal tissues and cancer cell lines), Panel 2 (containing samples derived from tissues from normal and cancer sources), Panel 3 (containing cancer cell lines), Panel 4 (containing cells and cell lines from normal tissues and cells related to inflammatory conditions), Panel 5D/5I (containing human tissues and cell lines with an emphasis on metabolic diseases), AI_comprehensive_panel (containing normal tissue and samples from autoinflammatory diseases), Panel CNSD.01 (containing samples from normal and diseased brains) and CNS_neurodegeneration_panel (containing samples from normal and diseased brains).

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs that would be indicative of degradation products. Samples are controlled against genomic DNA contamination by RTQ PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

First, the RNA samples were normalized to reference nucleic acids such as constitutively expressed genes (for example, β -actin and GAPDH). Normalized RNA (5 μ l) was converted to cDNA and analyzed by RTQ-PCR using One Step RT-PCR Master Mix Reagents (PE Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (T_m) range = 58°-60° C, primer optimal T_m = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe T_m must be 10° C greater than primer T_m , amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by SyntheGen (Houston, TX,

USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

5 PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl₂, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™
10 (PE Biosystems), and 0.4 U/μl RNase inhibitor, and 0.25 U/μl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given
15 sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100.

Panels 1, 1.1, 1.2, and 1.3D

20 The plates for Panels 1, 1.1, 1.2 and 1.3D include 2 control wells (genomic DNA control and chemistry control) and 94 wells containing cDNA from various samples. The samples in these panels are broken into 2 classes: samples derived from cultured cell lines and samples derived from primary normal tissues. The cell lines are derived from cancers of the following types: lung cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric
25 cancer and pancreatic cancer. Cell lines used in these panels are widely available through the American Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions recommended by the ATCC. The normal tissues found on these panels are comprised of samples derived from all major organ systems from single adult individuals or fetuses. These samples are derived from the following organs: adult skeletal
30 muscle, fetal skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph

node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose.

5 In the results for Panels 1, 1.1, 1.2 and 1.3D, the following abbreviations are used:

ca. = carcinoma,

* = established from metastasis,

met = metastasis,

s cell var = small cell variant,

10 non-s = non-sm = non-small,

squam = squamous,

pl. eff = pl effusion = pleural effusion,

glio = glioma,

astro = astrocytoma, and

15 neuro = neuroblastoma.

General_screening_panel_v1.4

The plates for Panel 1.4 include 2 control wells (genomic DNA control and chemistry control) and 94 wells containing cDNA from various samples. The samples in Panel 1.4 are broken into 2 classes: samples derived from cultured cell lines and samples derived from
20 primary normal tissues. The cell lines are derived from cancers of the following types: lung cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric cancer and pancreatic cancer. Cell lines used in Panel 1.4 are widely available through the American Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions
25 recommended by the ATCC. The normal tissues found on Panel 1.4 are comprised of pools of samples derived from all major organ systems from 2 to 5 different adult individuals or fetuses. These samples are derived from the following organs: adult skeletal muscle, fetal skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph node, pancreas,
30 salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose.

Panels 2D and 2.2

The plates for Panels 2D and 2.2 generally include 2 control wells and 94 test samples composed of RNA or cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins" obtained from noncancerous tissue just adjacent to the tumor. These are termed normal adjacent tissues and are denoted "NAT" in the results below. The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologists at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table RR). In addition, RNA and cDNA samples were obtained from various human tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissues were ascertained to be free of disease and were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics, and Invitrogen.

Panel 3D

The plates of Panel 3D are comprised of 94 cDNA samples and two control samples. Specifically, 92 of these samples are derived from cultured human cancer cell lines, 2 samples of human primary cerebellar tissue and 2 controls. The human cell lines are generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall into the following tissue groups: Squamous cell carcinoma of the tongue, breast cancer, prostate cancer, melanoma, epidermoid carcinoma, sarcomas, bladder carcinomas, pancreatic cancers, kidney cancers, leukemias/lymphomas, ovarian/uterine/cervical, gastric, colon, lung and CNS cancer cell lines. In addition, there are two independent samples of cerebellum. These cells are all cultured under standard recommended conditions and RNA extracted using the standard procedures. The cell lines in panel 3D and 1.3D are of the most common cell lines used in the scientific literature.

Panels 4D, 4R and 4.1D

Panel 4 includes samples on a 96 well plate (2 control wells, 94 test samples) composed of RNA (Panel 4R) or cDNA (Panels 4D/4.1D) isolated from various human cell lines or tissues related to inflammatory conditions. Total RNA from control normal tissues such as colon and lung (Stratagene, La Jolla, CA) and thymus and kidney (Clontech) were employed. Total RNA from liver tissue from cirrhosis patients and kidney from lupus patients was obtained from BioChain (Biochain Institute, Inc., Hayward, CA). Intestinal tissue for RNA preparation from patients diagnosed as having Crohn's disease and ulcerative colitis was obtained from the National Disease Research Interchange (NDRI) (Philadelphia, PA).

Astrocytes, lung fibroblasts, dermal fibroblasts, coronary artery smooth muscle cells, small airway epithelium, bronchial epithelium, microvascular dermal endothelial cells, microvascular lung endothelial cells, human pulmonary aortic endothelial cells, human umbilical vein endothelial cells were all purchased from Clonetics (Walkersville, MD) and grown in the media supplied for these cell types by Clonetics. These primary cell types were activated with various cytokines or combinations of cytokines for 6 and/or 12-14 hours, as indicated. The following cytokines were used; IL-1 beta at approximately 1-5 ng/ml, TNF alpha at approximately 5-10 ng/ml, IFN gamma at approximately 20-50 ng/ml, IL-4 at approximately 5-10 ng/ml, IL-9 at approximately 5-10 ng/ml, IL-13 at approximately 5-10 ng/ml. Endothelial cells were sometimes starved for various times by culture in the basal media from Clonetics with 0.1% serum.

Mononuclear cells were prepared from blood of employees at CuraGen Corporation, using Ficoll. LAK cells were prepared from these cells by culture in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco/Life Technologies, Rockville, MD), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and Interleukin 2 for 4-6 days. Cells were then either activated with 10-20 ng/ml PMA and 1-2 μ g/ml ionomycin, IL-12 at 5-10 ng/ml, IFN gamma at 20-50 ng/ml and IL-18 at 5-10 ng/ml for 6 hours. In some cases, mononuclear cells were cultured for 4-5 days in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco) with PHA (phytohemagglutinin) or PWM (pokeweed mitogen) at approximately 5 μ g/ml. Samples were taken at 24, 48 and 72 hours for RNA preparation. MLR (mixed lymphocyte reaction)

samples were obtained by taking blood from two donors, isolating the mononuclear cells using Ficoll and mixing the isolated mononuclear cells 1:1 at a final concentration of approximately 2×10^6 cells/ml in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol (5.5×10^{-5} M) (Gibco), and 10 mM Hepes (Gibco). The MLR was cultured and samples taken at various time points ranging from 1- 7 days for RNA preparation.

Monocytes were isolated from mononuclear cells using CD14 Miltenyi Beads, +ve VS selection columns and a Vario Magnet according to the manufacturer's instructions.

Monocytes were differentiated into dendritic cells by culture in DMEM 5% fetal calf serum (FCS) (Hyclone, Logan, UT), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco), 50 ng/ml GM-CSF and 5 ng/ml IL-4 for 5-7 days. Macrophages were prepared by culture of monocytes for 5-7 days in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), 10 mM Hepes (Gibco) and 10% AB Human Serum or MCSF at approximately 50 ng/ml. Monocytes, macrophages and dendritic cells were stimulated for 6 and 12-14 hours with lipopolysaccharide (LPS) at 100 ng/ml. Dendritic cells were also stimulated with anti-CD40 monoclonal antibody (Pharmingen) at 10 μ g/ml for 6 and 12-14 hours.

CD4 lymphocytes, CD8 lymphocytes and NK cells were also isolated from mononuclear cells using CD4, CD8 and CD56 Miltenyi beads, positive VS selection columns and a Vario Magnet according to the manufacturer's instructions. CD45RA and CD45RO CD4 lymphocytes were isolated by depleting mononuclear cells of CD8, CD56, CD14 and CD19 cells using CD8, CD56, CD14 and CD19 Miltenyi beads and positive selection. Then CD45RO beads were used to isolate the CD45RO CD4 lymphocytes with the remaining cells being CD45RA CD4 lymphocytes. CD45RA CD4, CD45RO CD4 and CD8 lymphocytes were placed in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and plated at 10^6 cells/ml onto Falcon 6 well tissue culture plates that had been coated overnight with 0.5 μ g/ml anti-CD28 (Pharmingen) and 3 μ g/ml anti-CD3 (OKT3, ATCC) in PBS. After 6 and 24 hours, the cells were harvested for RNA preparation. To prepare chronically activated CD8 lymphocytes, we activated the isolated CD8 lymphocytes for 4 days on anti-CD28 and anti-CD3 coated plates and then harvested the cells and expanded them in

DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and IL-2. The expanded CD8 cells were then activated again with plate bound anti-CD3 and anti-CD28 for 4 days and expanded as before. RNA was isolated 6 and 24 hours after the second activation and after 4 days of the second expansion culture. The isolated NK cells were cultured in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco) and IL-2 for 4-6 days before RNA was prepared.

To obtain B cells, tonsils were procured from NDRI. The tonsil was cut up with sterile dissecting scissors and then passed through a sieve. Tonsil cells were then spun down and resuspended at 10^6 cells/ml in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco). To activate the cells, we used PWM at 5 μ g/ml or anti-CD40 (Pharmingen) at approximately 10 μ g/ml and IL-4 at 5-10 ng/ml. Cells were harvested for RNA preparation at 24, 48 and 72 hours.

To prepare the primary and secondary Th1/Th2 and Tr1 cells, six-well Falcon plates were coated overnight with 10 μ g/ml anti-CD28 (Pharmingen) and 2 μ g/ml OKT3 (ATCC), and then washed twice with PBS. Umbilical cord blood CD4 lymphocytes (Poietic Systems, German Town, MD) were cultured at 10^5 - 10^6 cells/ml in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), 10 mM Hepes (Gibco) and IL-2 (4 ng/ml). IL-12 (5 ng/ml) and anti-IL4 (1 μ g/ml) were used to direct to Th1, while IL-4 (5 ng/ml) and anti-IFN gamma (1 μ g/ml) were used to direct to Th2 and IL-10 at 5 ng/ml was used to direct to Tr1. After 4-5 days, the activated Th1, Th2 and Tr1 lymphocytes were washed once in DMEM and expanded for 4-7 days in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), 10 mM Hepes (Gibco) and IL-2 (1 ng/ml). Following this, the activated Th1, Th2 and Tr1 lymphocytes were re-stimulated for 5 days with anti-CD28/OKT3 and cytokines as described above, but with the addition of anti-CD95L (1 μ g/ml) to prevent apoptosis. After 4-5 days, the Th1, Th2 and Tr1 lymphocytes were washed and then expanded again with IL-2 for 4-7 days. Activated Th1 and Th2 lymphocytes were maintained in this way for a maximum of three cycles. RNA was prepared

from primary and secondary Th1, Th2 and Tr1 after 6 and 24 hours following the second and third activations with plate bound anti-CD3 and anti-CD28 mAbs and 4 days into the second and third expansion cultures in Interleukin 2.

The following leukocyte cells lines were obtained from the ATCC: Ramos, EOL-1, KU-812. EOL cells were further differentiated by culture in 0.1 mM dbcAMP at 5×10^5 cells/ml for 8 days, changing the media every 3 days and adjusting the cell concentration to 5×10^5 cells/ml. For the culture of these cells, we used DMEM or RPMI (as recommended by the ATCC), with the addition of 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), 10 mM Hepes (Gibco). RNA was either prepared from resting cells or cells activated with PMA at 10 ng/ml and ionomycin at 1 μ g/ml for 6 and 14 hours. Keratinocyte line CCD106 and an airway epithelial tumor line NCI-H292 were also obtained from the ATCC. Both were cultured in DMEM 5% FCS (Hyclone), 100 μ M non essential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5} M (Gibco), and 10 mM Hepes (Gibco). CCD1106 cells were activated for 6 and 14 hours with approximately 5 ng/ml TNF alpha and 1 ng/ml IL-1 beta, while NCI-H292 cells were activated for 6 and 14 hours with the following cytokines: 5 ng/ml IL-4, 5 ng/ml IL-9, 5 ng/ml IL-13 and 25 ng/ml IFN gamma.

For these cell lines and blood cells, RNA was prepared by lysing approximately 10^7 cells/ml using Trizol (Gibco BRL). Briefly, 1/10 volume of bromochloropropane (Molecular Research Corporation) was added to the RNA sample, vortexed and after 10 minutes at room temperature, the tubes were spun at 14,000 rpm in a Sorvall SS34 rotor. The aqueous phase was removed and placed in a 15 ml Falcon Tube. An equal volume of isopropanol was added and left at -20 degrees C overnight. The precipitated RNA was spun down at 9,000 rpm for 15 min in a Sorvall SS34 rotor and washed in 70% ethanol. The pellet was redissolved in 300 μ l of RNase-free water and 35 μ l buffer (Promega) 5 μ l DTT, 7 μ l RNasin and 8 μ l DNase were added. The tube was incubated at 37 degrees C for 30 minutes to remove contaminating genomic DNA, extracted once with phenol chloroform and re-precipitated with 1/10 volume of 3 M sodium acetate and 2 volumes of 100% ethanol. The RNA was spun down and placed in RNase free water. RNA was stored at -80 degrees C.

Panel CNSD.01

The plates for Panel CNSD.01 include two control wells and 94 test samples comprised of cDNA isolated from postmortem human brain tissue obtained from the Harvard Brain Tissue Resource Center. Brains are removed from calvaria of donors between 4 and 24 hours after death, sectioned by neuroanatomists, and frozen at -80°C in liquid nitrogen vapor. All brains are sectioned and examined by neuropathologists to confirm diagnoses with clear associated neuropathology.

Disease diagnoses are taken from patient records. The panel contains two brains from each of the following diagnoses: Alzheimer's disease, Parkinson's disease, Huntington's disease, Progressive Supranuclear Palsy, Depression, and "Normal controls". Within each of these brains, the following regions are represented: cingulate gyrus, temporal pole, globus pallidus, substantia nigra, Brodman Area 4 (primary motor strip), Brodman Area 7 (parietal cortex), Brodman Area 9 (prefrontal cortex), and Brodman area 17 (occipital cortex). Not all brain regions are represented in all cases; e.g., Huntington's disease is characterized in part by neurodegeneration in the globus pallidus, thus this region is impossible to obtain from confirmed Huntington's cases. Likewise Parkinson's disease is characterized by degeneration of the substantia nigra making this region more difficult to obtain. Normal control brains were examined for neuropathology and found to be free of any pathology consistent with neurodegeneration.

In the labels employed to identify tissues in the CNS panel, the following abbreviations are used:

PSP = Progressive supranuclear palsy

Sub Nigra = Substantia nigra

Glob Pallidus= Globus pallidus

Temp Pole = Temporal pole

Cing Gyr = Cingulate gyrus

BA 4 = Brodman Area 4

Panel CNS_Neurodegeneration_V1.0

The plates for Panel CNS_Neurodegeneration_V1.0 include two control wells and 47 test samples comprised of cDNA isolated from postmortem human brain tissue obtained from the Harvard Brain Tissue Resource Center (McLean Hospital) and the Human Brain and

Spinal Fluid Resource Center (VA Greater Los Angeles Healthcare System). Brains are removed from calvaria of donors between 4 and 24 hours after death, sectioned by neuroanatomists, and frozen at -80°C in liquid nitrogen vapor. All brains are sectioned and examined by neuropathologists to confirm diagnoses with clear associated neuropathology.

5 Disease diagnoses are taken from patient records. The panel contains six brains from Alzheimer's disease (AD) patients, and eight brains from "Normal controls" who showed no evidence of dementia prior to death. The eight normal control brains are divided into two categories: Controls with no dementia and no Alzheimer's like pathology (Controls) and controls with no dementia but evidence of severe Alzheimer's like pathology, (specifically
10 senile plaque load rated as level 3 on a scale of 0-3; 0 = no evidence of plaques, 3 = severe AD senile plaque load). Within each of these brains, the following regions are represented: hippocampus, temporal cortex (Brodmann Area 21), parietal cortex (Brodmann area 7), and occipital cortex (Brodmann area 17). These regions were chosen to encompass all levels of neurodegeneration in AD. The hippocampus is a region of early and severe neuronal loss in
15 AD; the temporal cortex is known to show neurodegeneration in AD after the hippocampus; the parietal cortex shows moderate neuronal death in the late stages of the disease; the occipital cortex is spared in AD and therefore acts as a "control" region within AD patients. Not all brain regions are represented in all cases.

In the labels employed to identify tissues in the CNS_Neurodegeneration_V1.0 panel,
20 the following abbreviations are used:

AD = Alzheimer's disease brain; patient was demented and showed AD-like pathology upon autopsy

Control = Control brains; patient not demented, showing no neuropathology

Control (Path) = Control brains; patient not demented but showing severe AD-like
25 pathology

SupTemporal Ctx = Superior Temporal Cortex

Inf Temporal Ctx = Inferior Temporal Cortex

GPCR1 (also referred to as GMAC073079_A, AC073079_da1, AC073079_da3 / CG109854-01, BA113A10_B_da1, BA113A10_B_da3 and CG50303-02)

Expression of the GPCR1 gene (i.e., GMAC073079_A and variants AC073079_da1, AC073079_da3, BA113A10_B_da1, BA113A10_B_da3, and CG50303-02) was assessed using the primer-probe sets Ag2610, Ag2607, Ag1585, Ag1501, Ag2545, and Ag2377 described in Tables 15A, 15B, 15C, 15D, and 15E. Please note that Ag2545 contains a single mismatch in the forward primer relative to the GMAC073079_A, AC073079_da1, AC073079_da3, BA113A10_B_da1, and BA113A10_B_da3 sequences. These mismatches are not predicted to alter the RTQ-PCR results. Results from RTQ-PCR runs are shown in Tables 15F, 15G, 15H, 15I, 15J, 15K, and 15L.

Table 15A. Probe Name Ag2610 (SEQ ID NO: 126, 127, 128)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GTCTCACCTCACACTGGTCTTC-3'	58.8	22	808
Probe	TET-5'-CATCTTCTGTATGTCAGGCCTGGCA-3'-TAMRA	69.3	26	847
Reverse	5'-CTGACTTGACAGAGTGAGCTT-3'	59.9	22	873

Table 15B. Probe Name Ag2607 (SEQ ID NO: 129, 130, 131)

Primers	Sequences	TM	Length	Start Position
Forward	5'-CATAGCTGACACCCACCTACAT-3'	59	22	180
Probe	FAM-5'-CACCCATGTACTTCTTCCTGGGCAAT-3'-TAMRA	68.9	26	203
Reverse	5'-ACTGCAGTCATGGTTACCAAGA-3'	59.7	22	245

Table 15C. Probe Names Ag1585/1501 (identical sequences) (SEQ ID NO: 132, 133, 134)

Primers	Sequences	TM	Length	Start Position
Forward	5'-CATAGCTGACACCCACCTACAT-3'	59	22	159
Probe	TET-5'-CACCCATGTACTTCTTCCTGGGCAAT-3'-TAMRA	68.9	26	182
Reverse	5'-CTGCAGTCATGGTTACCAAGAT-3'	59.1	22	223

Table 15D. Probe Name Ag2545 (SEQ ID NO: 135, 136, 137)

Primers	Sequences	TM	Length	Start Position
Forward	5'-AAGGCCTTTCAGCCTCTACA-3'	59.1	20	22
Probe	FAM-5'-TCTGCCCCGTAGCACTGTTTAACTG-3'-TAMRA	67.4	27	42
Reverse	5'-CCCCTTCTCAATCCCTTTAT-3'	58.5	21	89

Table 15E. Probe Name Ag2377 (SEQ ID NO: 138, 139, 140)

Primers	Sequences	TM	Length	Start Position
Forward	5'-ATGGGAAACACCATCATCATAG-3'	58.7	22	135
Probe	TET-5'-TGATCATAGCTGACACCCACCTACAT-3'-TAMRA	66.8	26	160
Reverse	5'-AATTGCCAGGAAGAAGTACAT-3'	59	22	192

Table 15F. Panel 1.2

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	1.2tm2087t_ag1501		1.2tm2087t_ag1501
Endothelial cells	6.8	Renal ca. 786-0	14.3
Heart (fetal)	0.3	Renal ca. A498	12.6
Pancreas	0.6	Renal ca. RXF 393	15.9
Pancreatic ca. CAPAN 2	0.3	Renal ca. ACHN	11.8
Adrenal Gland (new lot*)	16.4	Renal ca. UO-31	21.0
Thyroid	0.0	Renal ca. TK-10	28.3
Salivary gland	38.2	Liver	7.6
Pituitary gland	1.2	Liver (fetal)	5.1
Brain (fetal)	20.6	Liver ca. (hepatoblast) HepG2	9.9
Brain (whole)	13.3	Lung	0.0
Brain (amygdala)	17.2	Lung (fetal)	0.9
Brain (cerebellum)	7.0	Lung ca. (small cell) LX-1	33.9
Brain (hippocampus)	67.4	Lung ca. (small cell) NCI-H69	58.6
Brain (thalamus)	92.0	Lung ca. (s.cell var.) SHP-77	3.6
Cerebral Cortex	85.3	Lung ca. (large cell) NCI-H460	24.0
Spinal cord	25.0	Lung ca. (non-sm. cell) A549	30.8
CNS ca. (glio/astro) U87-MG	17.0	Lung ca. (non-s.cell) NCI-H23	81.8
CNS ca. (glio/astro) U-118-MG	5.5	Lung ca (non-s.cell) HOP-62	24.3
CNS ca. (astro) SW1783	7.2	Lung ca. (non-s.cl) NCI-H522	59.0
CNS ca.* (neuro; met) SK-N-AS	1.5	Lung ca. (squam.) SW 900	10.4
CNS ca. (astro) SF-539	2.4	Lung ca. (squam.) NCI-H596	25.7
CNS ca. (astro) SNB-75	14.2	Mammary gland	15.3
CNS ca. (glio) SNB-19	23.5	Breast ca.* (pl. effusion) MCF-7	2.1
CNS ca. (glio) U251	6.9	Breast ca.* (pl.ef) MDA-MB-231	2.3
CNS ca. (glio) SF-295	18.8	Breast ca.* (pl. effusion) T47D	88.9
Heart	50.7	Breast ca. BT-549	6.1
Skeletal Muscle (new lot*)	13.2	Breast ca. MDA-N	74.7
Bone marrow	7.5	Ovary	0.7
Thymus	0.0	Ovarian ca. OVCAR-3	14.3
Spleen	4.6	Ovarian ca. OVCAR-4	40.3
Lymph node	2.9	Ovarian ca. OVCAR-5	72.7
Colorectal	9.3	Ovarian ca. OVCAR-8	100.0
Stomach	0.7	Ovarian ca. IGROV-1	56.3

Small intestine	6.5	Ovarian ca.* (ascites) SK-OV-3	16.4
Colon ca. SW480	1.5	Uterus	9.5
Colon ca.* (SW480 met)SW620	16.2	Placenta	39.0
Colon ca. HT29	14.7	Prostate	8.8
Colon ca. HCT-116	7.6	Prostate ca.* (bone met)PC-3	39.0
Colon ca. CaCo-2	9.4	Testis	14.0
83219 CC Well to Mod Diff (ODO3866)	31.2	Melanoma Hs688(A).T	1.3
Colon ca. HCC-2998	10.3	Melanoma* (met) Hs688(B).T	10.6
Gastric ca.* (liver met) NCI-N87	21.3	Melanoma UACC-62	48.6
Bladder	36.6	Melanoma M14	69.7
Trachea	0.0	Melanoma LOX IMVI	0.0
Kidney	35.8	Melanoma* (met) SK-MEL-5	9.7
Kidney (fetal)	14.9		

Table 15G. Panel 1.3D

Tissue Name	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)
	1.3dx4tm5649t ag2610 a2	1.3dx4tm5657f ag2607 b1	1.3dx4tm5389t ag1585 a2	1.3dx4tm5454t ag2377 b1
Liver adenocarcinoma	0.0	0.0	5.1	5.6
Pancreas	0.0	0.0	0.0	0.0
Pancreatic ca. CAPAN 2	5.6	0.0	0.0	0.0
Adrenal gland	0.0	0.0	0.0	0.0
Thyroid	0.0	0.0	0.0	0.0
Salivary gland	0.0	7.0	11.0	4.6
Pituitary gland	29.0	7.7	0.0	0.0
Brain (fetal)	21.0	12.7	31.2	53.2
Brain (whole)	100.0	100.0	56.4	48.7
Brain (amygdala)	0.0	31.9	15.4	0.0
Brain (cerebellum)	25.3	0.0	10.0	19.5
Brain (hippocampus)	7.0	17.3	5.5	20.3
Brain (substantia nigra)	31.8	49.3	54.4	80.8
Brain (thalamus)	72.3	55.4	26.2	58.1
Cerebral Cortex	0.0	14.2	5.6	0.0
Spinal cord	38.8	98.6	100.0	100.0
CNS ca. (glio/astro) U87-MG	0.0	0.0	13.0	0.0
CNS ca. (glio/astro) U-118-MG	0.0	0.0	4.2	7.2
CNS ca. (astro) SW1783	0.0	19.8	0.0	0.0
CNS ca.* (neuro; met) SK-N-AS	0.0	0.0	0.0	0.0

CNS ca. (astro) SF-539	5.4	0.0	12.4	6.5
CNS ca. (astro) SNB-75	3.9	0.0	0.0	0.0
CNS ca. (glio) SNB-19	18.2	4.5	2.1	4.0
CNS ca. (glio) U251	0.0	0.0	19.4	0.0
CNS ca. (glio) SF-295	0.0	0.0	23.4	0.0
Heart (fetal)	0.0	0.0	0.0	0.0
Heart	0.0	0.0	12.5	10.7
Fetal Skeletal	0.0	0.0	0.0	0.0
Skeletal muscle	0.0	0.0	6.1	0.0
Bone marrow	0.0	0.0	0.0	0.0
Thymus	0.0	0.0	0.0	0.0
Spleen	0.0	0.0	0.0	0.0
Lymph node	0.0	0.0	6.7	0.0
Colorectal	0.0	0.0	10.7	4.9
Stomach	7.2	0.0	0.0	0.0
Small intestine	0.0	0.0	0.0	0.0
Colon ca. SW480	5.2	0.0	0.0	0.0
Colon ca.* (SW480 met)SW620	8.5	6.8	6.8	0.0
Colon ca. HT29	0.0	0.0	0.0	0.0
Colon ca. HCT-116	0.0	0.0	5.9	0.0
Colon ca. CaCo-2	0.0	0.0	0.0	6.6
83219 CC Well to Mod Diff (ODO3866)	0.0	0.0	0.0	0.0
Colon ca. HCC-2998	0.0	0.0	0.0	0.0
Gastric ca.* (liver met) NCI- N87	8.0	0.0	0.0	6.7
Bladder	17.5	9.8	2.8	0.0
Trachea	0.0	0.0	0.0	6.4
Kidney	0.0	0.0	0.0	0.0
Kidney (fetal)	12.4	0.0	0.0	0.0
Renal ca. 786-0	0.0	0.0	0.0	3.4
Renal ca. A498	6.1	0.0	6.9	0.0
Renal ca. RXF 393	7.9	0.0	0.0	9.6
Renal ca. ACHN	6.4	3.1	0.0	0.0
Renal ca. UO-31	0.0	0.0	11.0	0.0
Renal ca. TK-10	0.0	0.0	4.9	0.0
Liver	0.0	0.0	8.0	0.0
Liver (fetal)	0.0	0.0	0.0	0.0
Liver ca. (hepatoblast) HepG2	0.0	0.0	0.0	0.0
Lung	0.0	0.0	10.9	0.0
Lung (fetal)	0.0	0.0	0.0	0.0
Lung ca. (small cell) LX-1	0.0	7.4	3.5	14.0
Lung ca. (small cell) NCI-H69	0.0	0.0	0.0	0.0

Lung ca. (s.cell var.) SHP-77	4.1	0.0	0.0	0.0
Lung ca. (large cell) NCI-H460	0.0	0.0	3.3	0.0
Lung ca. (non-sm. cell) A549	0.0	0.0	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	5.6	8.3	5.2	14.3
Lung ca (non-s.cell) HOP-62	0.0	0.0	0.0	0.0
Lung ca. (non-s.cl) NCI-H522	0.0	0.0	0.0	0.0
Lung ca. (squam.) SW 900	0.0	0.0	7.2	5.4
Lung ca. (squam.) NCI-H596	0.0	0.0	0.0	0.0
Mammary gland	0.0	7.7	0.0	0.0
Breast ca.* (pl. effusion) MCF-7	0.0	0.0	4.7	0.0
Breast ca.* (pl.ef) MDA-MB-231	6.0	0.0	0.0	0.0
Breast ca.* (pl. effusion) T47D	29.3	6.9	13.9	0.0
Breast ca. BT-549	0.0	0.0	0.0	0.0
Breast ca. MDA-N	0.0	18.0	5.2	12.6
Ovary	0.0	0.0	0.0	0.0
Ovarian ca. OVCAR-3	0.0	15.9	0.0	0.0
Ovarian ca. OVCAR-4	5.0	0.0	0.0	0.0
Ovarian ca. OVCAR-5	4.5	11.7	7.7	0.0
Ovarian ca. OVCAR-8	8.0	18.2	29.7	15.9
Ovarian ca. IGROV-1	0.0	0.0	17.5	0.0
Ovarian ca.* (ascites) SK-OV-3	6.4	0.0	0.0	0.0
Uterus	0.0	6.1	0.0	9.6
Placenta	39.0	43.2	51.8	21.5
Prostate	0.0	0.0	0.0	14.6
Prostate ca.* (bone met)PC-3	0.0	5.9	0.0	0.0
Testis	10.5	18.5	17.4	14.7
Melanoma Hs688(A).T	0.0	0.0	0.0	0.0
Melanoma* (met) Hs688(B).T	0.0	0.0	0.0	0.0
Melanoma UACC-62	6.9	0.0	0.0	3.7
Melanoma M14	0.0	12.1	6.4	35.6
Melanoma LOX IMVI	0.0	0.0	0.0	0.0
Melanoma* (met) SK-MEL-5	10.4	0.0	0.0	0.0
Adipose	0.0	0.0	0.0	0.0

Table 15H. Panel 2.2

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	2.2x4tm6456t ag2377_b1		2.2x4tm6456t ag2377_b1
Normal Colon GENPAK 061003	0.0	83793 Kidney NAT (OD04348)	0.0

97759 Colon cancer (OD06064)	15.3	98938 Kidney malignant cancer (OD06204B)	24.2
97760 Colon cancer NAT (OD06064)	0.0	98939 Kidney normal adjacent tissue (OD06204E)	0.0
97778 Colon cancer (OD06159)	0.0	85973 Kidney Cancer (OD04450-01)	0.0
97779 Colon cancer NAT (OD06159)	0.0	85974 Kidney NAT (OD04450-03)	0.0
98861 Colon cancer (OD06297-04)	0.0	Kidney Cancer Clontech 8120613	0.0
98862 Colon cancer NAT (OD06297-015)	0.0	Kidney NAT Clontech 8120614	0.0
83237 CC Gr.2 ascend colon (ODO3921)	0.0	Kidney Cancer Clontech 9010320	0.0
83238 CC NAT (ODO3921)	0.0	Kidney NAT Clontech 9010321	0.0
97766 Colon cancer metastasis (OD06104)	0.0	Kidney Cancer Clontech 8120607	0.0
97767 Lung NAT (OD06104)	0.0	Kidney NAT Clontech 8120608	0.0
87472 Colon mets to lung (OD04451-01)	27.9	Normal Uterus GENPAK 061018	35.7
87473 Lung NAT (OD04451-02)	32.5	Uterus Cancer GENPAK 064011	0.0
Normal Prostate Clontech A+ 6546-1 (8090438)	0.0	Normal Thyroid Clontech A+ 6570-1 (7080817)	0.0
84140 Prostate Cancer (OD04410)	0.0	Thyroid Cancer GENPAK 064010	0.0
84141 Prostate NAT (OD04410)	0.0	Thyroid Cancer INVITROGEN A302152	0.0
Normal Ovary Res. Gen.	0.0	Thyroid NAT INVITROGEN A302153	0.0
98863 Ovarian cancer (OD06283-03)	10.7	Normal Breast GENPAK 061019	14.9
98865 Ovarian cancer NAT/fallopian tube (OD06283-07)	0.0	84877 Breast Cancer (OD04566)	0.0
Ovarian Cancer GENPAK 064008	0.0	Breast Cancer Res. Gen. 1024	84.1
97773 Ovarian cancer (OD06145)	0.0	85975 Breast Cancer (OD04590-01)	0.0
97775 Ovarian cancer NAT (OD06145)	34.9	85976 Breast Cancer Mets (OD04590-03)	22.6
98853 Ovarian cancer (OD06455-03)	24.7	87070 Breast Cancer Metastasis (OD04655-05)	0.0
98854 Ovarian NAT (OD06455-07) Fallopian tube	9.9	GENPAK Breast Cancer 064006	0.0
Normal Lung GENPAK 061010	0.0	Breast Cancer Clontech 9100266	100.0
92337 Invasive poor diff. lung adeno (ODO4945-01)	12.7	Breast NAT Clontech 9100265	9.9

92338 Lung NAT (ODO4945-03)	0.0	Breast Cancer INVITROGEN A209073	0.0
84136 Lung Malignant Cancer (OD03126)	0.0	Breast NAT INVITROGEN A2090734	14.6
84137 Lung NAT (OD03126)	0.0	97763 Breast cancer (OD06083)	75.8
90372 Lung Cancer (OD05014A)	0.0	97764 Breast cancer node metastasis (OD06083)	16.6
90373 Lung NAT (OD05014B)	19.5	Normal Liver GENPAK 061009	0.0
97761 Lung cancer (OD06081)	25.9	Liver Cancer Research Genetics RNA 1026	0.0
97762 Lung cancer NAT (OD06081)	0.0	Liver Cancer Research Genetics RNA 1025	33.4
85950 Lung Cancer (OD04237-01)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.0
85970 Lung NAT (OD04237-02)	0.0	Paired Liver Tissue Research Genetics RNA 6004-N	0.0
83255 Ocular Mel Met to Liver (ODO4310)	13.6	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0
83256 Liver NAT (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
84139 Melanoma Mets to Lung (OD04321)	0.0	Liver Cancer GENPAK 064003	0.0
84138 Lung NAT (OD04321)	0.0	Normal Bladder GENPAK 061001	0.0
Normal Kidney GENPAK 061008	18.0	Bladder Cancer Research Genetics RNA 1023	0.0
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	Bladder Cancer INVITROGEN A302173	0.0
83787 Kidney NAT (OD04338)	0.0	Normal Stomach GENPAK 061017	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	15.4	Gastric Cancer Clontech 9060397	0.0
83789 Kidney NAT (OD04339)	0.0	NAT Stomach Clontech 9060396	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	Gastric Cancer Clontech 9060395	33.3
83791 Kidney NAT (OD04340)	0.0	NAT Stomach Clontech 9060394	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Gastric Cancer GENPAK 064005	0.0

Table 15I. Panel 4D

Tissue Name	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)
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	4dtm5333t_ ag1585	4Dx4tm4622t_ ag2377_b1	4dx4tm4955f_ ag2607_b2
93768 Secondary Th1 anti-CD28/anti-CD3	0.0	27.4	14.2
93769 Secondary Th2 anti-CD28/anti-CD3	0.0	36.5	0.0
93770 Secondary Tr1 anti-CD28/anti-CD3	0.0	10.5	9.4
93573 Secondary Th1 resting day 4-6 in IL-2	0.0	0.0	0.0
93572 Secondary Th2 resting day 4-6 in IL-2	0.0	0.0	0.0
93571 Secondary Tr1 resting day 4-6 in IL-2	0.0	12.9	0.0
93568 primary Th1 anti-CD28/anti-CD3	0.0	0.0	0.0
93569 primary Th2 anti-CD28/anti-CD3	0.0	8.1	0.0
93570 primary Tr1 anti-CD28/anti-CD3	0.0	25.4	10.3
93565 primary Th1 resting dy 4-6 in IL-2	0.0	28.2	53.1
93566 primary Th2 resting dy 4-6 in IL-2	0.0	69.3	23.3
93567 primary Tr1 resting dy 4-6 in IL-2	0.0	13.3	15.1
93351 CD45RA CD4 lymphocyte anti-CD28/anti-CD3	0.0	0.0	0.0
93352 CD45RO CD4 lymphocyte anti-CD28/anti-CD3	0.0	34.4	7.3
93251 CD8 Lymphocytes anti-CD28/anti-CD3	0.0	8.7	9.2
93353 chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	100.0	31.1	13.1
93574 chronic CD8 Lymphocytes 2ry_activated CD3/CD28	0.0	12.3	10.8
93354 CD4 none	0.0	0.0	0.0
93252 Secondary Th1/Th2/Tr1 anti-CD95 CH11	0.0	25.9	14.6
93103 LAK cells resting	0.0	28.5	33.1
93788 LAK cells IL-2	0.0	0.0	9.9
93787 LAK cells IL-2+IL-12	0.0	20.1	0.0
93789 LAK cells IL-2+IFN gamma	0.0	12.2	10.0
93790 LAK cells IL-2+ IL-18	0.0	9.4	0.0
93104 LAK cells PMA/ionomycin and IL-18	0.0	19.0	0.0
93578 NK Cells IL-2 resting	0.0	25.5	17.5
93109 Mixed Lymphocyte Reaction_Two Way MLR	0.0	31.3	5.4
93110 Mixed Lymphocyte Reaction_Two Way MLR	0.0	0.0	12.0
93111 Mixed Lymphocyte Reaction_Two Way MLR	0.0	10.6	0.0
93112 Mononuclear Cells (PBMCs) resting	0.0	11.5	5.0
93113 Mononuclear Cells (PBMCs) PWM	0.0	12.3	19.5
93114 Mononuclear Cells (PBMCs) PHA-L	0.0	28.3	18.6
93249 Ramos (B cell) none	0.0	13.9	18.8
93250 Ramos (B cell) ionomycin	0.0	16.7	7.8
93349 B lymphocytes PWM	0.0	15.7	12.9

93350 B lymphocytes CD40L and IL-4	0.0	24.8	0.0
92665_EOL-1 (Eosinophil)_dbcAMP differentiated	0.0	13.7	7.4
93248 EOL-1 (Eosinophil) dbcAMP/PMAionomycin	0.0	6.2	0.0
93356 Dendritic Cells_none	0.0	15.6	29.5
93355 Dendritic Cells_LPS 100 ng/ml	0.0	10.7	17.0
93775 Dendritic Cells_anti-CD40	0.0	11.6	5.6
93774 Monocytes_resting	0.0	0.0	0.0
93776 Monocytes_LPS 50 ng/ml	0.0	100.0	50.3
93581 Macrophages_resting	0.1	77.3	100.0
93582 Macrophages_LPS 100 ng/ml	0.0	8.0	8.4
93098 HUVEC (Endothelial)_none	0.0	9.0	0.0
93099 HUVEC (Endothelial)_starved	0.0	41.7	2.8
93100 HUVEC (Endothelial)_IL-1b	0.0	0.0	0.0
93779 HUVEC (Endothelial)_IFN gamma	0.0	0.0	4.0
93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	0.0	0.0	4.0
93101 HUVEC (Endothelial)_TNF alpha + IL4	0.0	12.4	6.7
93781 HUVEC (Endothelial)_IL-11	0.0	12.6	0.0
93583_Lung Microvascular Endothelial Cells_none	0.0	18.8	16.8
93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	12.9
92662_Microvascular Dermal endothelium_none	0.0	35.1	4.5
92663_Microvascular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	8.3	10.8
93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.0	0.0	0.0
93347_Small Airway Epithelium_none	0.0	0.0	0.0
93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	10.7
92668_Coronary Artery SMC_resting	0.0	12.3	0.0
92669_Coronary Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	0.0
93107_astrocytes_resting	0.0	19.1	5.1
93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	8.9	0.0
92666_KU-812 (Basophil)_resting	0.0	0.0	0.0
92667_KU-812 (Basophil)_PMA/ionomycin	0.0	13.9	5.6
93579_CCD1106 (Keratinocytes)_none	0.0	10.8	4.3
93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	0.0	12.7	0.0
93791_Liver Cirrhosis	0.0	82.2	50.1

93792_Lupus Kidney	0.0	23.2	8.6
93577_NCI-H292	0.0	0.0	3.7
93358_NCI-H292_IL-4	0.0	44.6	0.0
93360_NCI-H292_IL-9	0.0	7.0	0.0
93359_NCI-H292_IL-13	0.0	0.0	3.9
93357_NCI-H292_IFN gamma	0.0	11.0	1.9
93777_HPAEC_-	0.0	6.3	11.3
93778_HPAEC_IL-1 beta/TNA alpha	0.0	24.4	4.5
93254_Normal Human Lung Fibroblast_none	0.0	0.0	0.0
93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0	0.0	0.0
93257_Normal Human Lung Fibroblast_IL-4	0.0	0.0	0.0
93256_Normal Human Lung Fibroblast_IL-9	0.0	0.0	3.4
93255_Normal Human Lung Fibroblast_IL-13	5.1	0.0	0.0
93258_Normal Human Lung Fibroblast_IFN gamma	0.0	0.0	5.1
93106_Dermal Fibroblasts CCD1070_resting	0.0	2.4	15.7
93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	0.0	66.4	10.5
93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	0.0	0.0	0.0
93772_dermal fibroblast_IFN gamma	0.0	15.1	7.5
93771_dermal fibroblast_IL-4	0.0	0.0	0.0
93260_IBD Colitis 2	0.0	10.7	0.0
93261_IBD Crohns	0.0	0.0	0.0
735010_Colon_normal	0.0	0.0	0.0
735019_Lung_none	0.0	0.0	4.5
64028-1_Thymus_none	0.0	24.2	13.7
64030-1_Kidney_none	0.0	44.6	17.7

Table 15J. Panel CNS_1

Tissue Name	Relative Expression(%)	
	cns1x4tm6185t ag2377_a2	cns1tm6570t ag2377
102633_BA4 Control	6.2	7.4
102641_BA4 Control2	8.8	4.2
102625_BA4 Alzheimer's2	0.0	7.1
102649_BA4 Parkinson's	24.7	19.8
102656_BA4 Parkinson's2	17.8	30.8
102664_BA4 Huntington's	17.6	3.7
102671_BA4 Huntington's2	8.1	0.0
102603_BA4 PSP	38.3	12.2
102610_BA4 PSP2	20.1	5.2

102588 BA4 Depression	49.7	31.2
102596 BA4 Depression2	14.2	18.0
102634 BA7 Control	23.4	2.7
102642 BA7 Control2	25.6	11.5
102626 BA7 Alzheimer's2	19.0	4.4
102650 BA7 Parkinson's	11.5	9.8
102657 BA7 Parkinson's2	0.0	14.6
102665 BA7 Huntington's	23.7	10.9
102672 BA7 Huntington's2	43.1	26.8
102604 BA7 PSP	30.9	14.6
102611 BA7 PSP2	4.2	10.4
102589 BA7 Depression	32.0	21.3
102632 BA9 Control	2.0	4.4
102640 BA9 Control2	16.7	24.7
102617 BA9 Alzheimer's	0.0	6.6
102624 BA9 Alzheimer's2	2.9	0.0
102648 BA9 Parkinson's	11.4	15.2
102655 BA9 Parkinson's2	8.0	4.2
102663 BA9 Huntington's	39.8	14.5
102670 BA9 Huntington's2	8.2	3.7
102602 BA9 PSP	44.5	5.7
102609 BA9 PSP2	0.0	0.0
102587 BA9 Depression	15.1	5.9
102595 BA9 Depression2	14.4	8.7
102635 BA17 Control	47.1	30.4
102643 BA17 Control2	28.8	5.4
102627 BA17 Alzheimer's2	7.5	7.1
102651 BA17 Parkinson's	38.2	68.3
102658 BA17 Parkinson's2	24.0	9.3
102666 BA17 Huntington's	36.2	13.8
102673 BA17 Huntington's2	15.2	16.4
102590 BA17 Depression	58.7	27.7
102597 BA17 Depression2	65.6	60.3
102605 BA17 PSP	0.0	21.0
102612 BA17 PSP2	11.1	10.4
102637 Sub Nigra Control	42.6	41.2
102645 Sub Nigra Control2	29.5	3.6
102629 Sub Nigra Alzheimer's2	28.6	12.6
102660 Sub Nigra Parkinson's2	55.3	61.1
102667 Sub Nigra Huntington's	100.0	100.0
102674 Sub Nigra Huntington's2	17.4	21.2
102614 Sub Nigra PSP2	9.7	5.4

102592_Sub Nigra Depression	87.3	42.0
102599_Sub Nigra Depression2	33.0	20.4
102636_Glob Palladus Control	28.5	25.7
102644_Glob Palladus Control2	25.3	15.2
102620_Glob Palladus Alzheimer's	11.9	16.4
102628_Glob Palladus Alzheimer's2	4.2	36.9
102652_Glob Palladus Parkinson's	38.1	44.4
102659_Glob Palladus Parkinson's2	9.1	26.1
102606_Glob Palladus PSP	48.0	33.4
102613_Glob Palladus PSP2	10.8	9.9
102591_Glob Palladus Depression	41.0	39.5
102638_Temp Pole Control	0.0	0.0
102646_Temp Pole Control2	11.9	7.9
102622_Temp Pole Alzheimer's	0.0	0.0
102630_Temp Pole Alzheimer's2	0.0	3.4
102653_Temp Pole Parkinson's	17.3	7.1
102661_Temp Pole Parkinson's2	0.0	9.5
102668_Temp Pole Huntington's	0.0	4.9
102607_Temp Pole PSP	6.7	6.2
102615_Temp Pole PSP2	0.0	0.0
102600_Temp Pole Depression2	0.0	23.8
102639_Cing Gyr Control	31.3	27.4
102647_Cing Gyr Control2	16.8	24.5
102623_Cing Gyr Alzheimer's	17.8	13.2
102631_Cing Gyr Alzheimer's2	13.9	3.4
102654_Cing Gyr Parkinson's	26.4	30.8
102662_Cing Gyr Parkinson's2	24.9	25.9
102669_Cing Gyr Huntington's	31.0	28.7
102676_Cing Gyr Huntington's2	20.8	14.2
102608_Cing Gyr PSP	90.2	76.3
102616_Cing Gyr PSP2	0.0	20.3
102594_Cing Gyr Depression	52.7	61.1
102601_Cing Gyr Depression2	43.7	15.3

Table 15K. Panel CNS_1.1

Tissue Name	Relative Expression(%)	
	cns_1.1tm673 3t_ag2377_b1	cns_1.1tm673 4t_ag2377_b1
102601_Cing Gyr Depression2	39.2	13.9
102594_Cing Gyr Depression	35.7	23.2
102616_Cing Gyr PSP2	6.2	2.8
102608_Cing Gyr PSP	100.0	100.0

102676_Cing Gyr Huntington's2	32.4	10.5
102669_Cing Gyr Huntington's	27.2	8.7
102662_Cing Gyr Parkinson's2	12.7	1.9
102654_Cing Gyr Parkinson's	47.6	32.6
102631_Cing Gyr Alzheimer's2	0.0	7.2
102623_Cing Gyr Alzheimer's	13.8	3.6
102647_Cing Gyr Control2	77.5	1.4
102639_Cing Gyr Control	30.0	11.7
102600_Temp Pole Depression2	0.0	14.7
102615_Temp Pole PSP2	0.0	4.5
102607_Temp Pole PSP	5.5	3.3
102668_Temp Pole Huntington's	0.0	7.7
102661_Temp Pole Parkinson's2	0.0	0.0
102653_Temp Pole Parkinson's	27.5	4.9
102630_Temp Pole Alzheimer's2	0.0	0.0
102622_Temp Pole Alzheimer's	0.0	0.0
102646_Temp Pole Control2	21.3	8.4
102638_Temp Pole Control	0.0	0.0
102591_Glob Palladus Depression	35.7	16.4
102613_Glob Palladus PSP2	5.5	5.0
102606_Glob Palladus PSP	23.7	8.6
102659_Glob Palladus Parkinson's2	34.1	6.5
102652_Glob Palladus Parkinson's	16.3	20.3
102628_Glob Palladus Alzheimer's2	19.5	3.4
102620_Glob Palladus Alzheimer's	24.3	6.7
102644_Glob Palladus Control2	13.8	2.8
102636_Glob Palladus Control	33.2	17.6
102599_Sub Nigra Depression2	36.2	5.5
102592_Sub Nigra Depression	52.5	10.4
102614_Sub Nigra PSP2	12.4	15.9
102674_Sub Nigra Huntington's2	8.7	5.9
102667_Sub Nigra Huntington's	82.1	51.1
102660_Sub Nigra Parkinson's2	34.8	12.5
102629_Sub Nigra Alzheimer's2	34.0	15.0
102645_Sub Nigra Control2	6.3	5.3
102637_Sub Nigra Control	58.7	10.2
102597_BA17 Depression2	39.1	9.3
102590_BA17 Depression	43.4	50.8
102612_BA17 PSP2	5.3	11.8
102605_BA17 PSP	4.2	13.1
102673_BA17 Huntington's2	17.7	10.1
102666_BA17 Huntington's	36.8	6.9

102658_BA17 Parkinson's2	19.2	12.1
102651_BA17 Parkinson's	37.2	19.0
102627_BA17 Alzheimer's2	7.7	0.0
102643_BA17 Control2	35.9	20.0
102635_BA17 Control	35.1	22.7
102595_BA9 Depression2	8.6	3.8
102587_BA9 Depression	0.0	14.0
102609_BA9 PSP2	3.6	12.4
102602_BA9 PSP	48.6	18.3
102670_BA9 Huntington's2	6.9	5.0
102663_BA9 Huntington's	58.8	8.4
102655_BA9 Parkinson's2	0.0	2.5
102648_BA9 Parkinson's	7.8	0.0
102624_BA9 Alzheimer's2	0.0	0.0
102617_BA9 Alzheimer's	0.0	0.0
102640_BA9 Control2	26.4	12.2
102632_BA9 Control	15.1	0.0
102589_BA7 Depression	29.3	11.1
102611_BA7 PSP2	28.7	2.9
102604_BA7 PSP	7.0	6.5
102672_BA7 Huntington's2	18.5	23.6
102665_BA7 Huntington's	11.2	6.7
102657_BA7 Parkinson's2	0.0	0.0
102650_BA7 Parkinson's	9.5	1.2
102626_BA7 Alzheimer's2	19.6	0.0
102642_BA7 Control2	25.3	2.4
102634_BA7 Control	10.1	9.6
102596_BA4 Depression2	27.5	16.0
102588_BA4 Depression	10.8	15.6
102610_BA4 PSP2	15.2	16.9
102603_BA4 PSP	11.3	10.7
102671_BA4 Huntington's2	0.0	0.0
102664_BA4 Huntington's	0.0	3.8
102656_BA4 Parkinson's2	18.7	11.8
102649_BA4 Parkinson's	53.7	3.2
102625_BA4 Alzheimer's2	6.1	0.0
102641_BA4 Control2	0.0	4.2
102633_BA4 Control	35.5	4.7

Table 15L. Panel CNS_Neurodegeneration_v1.0

Tissue Name	Relative Expression (%)	Relative Expression (%)	Relative Expression (%)	Relative Expression (%)
	tm7016t_ ag2377 a2 s2	tm7022t_ ag2610 b1	tm7047f_ ag2607 a1 s2	tm6942f_ ag2545 b1
AD 1 Hippo	9.2	14.9	4.5	3.8
AD 2 Hippo	10.7	45.6	15.1	7.7
AD 3 Hippo	8.8	18.6	8.5	0.0
AD 4 Hippo	7.2	4.8	9.0	0.0
AD 5 hippo	32.6	47.9	44.3	35.8
AD 6 Hippo	100.0	33.8	35.5	8.4
Control 2 Hippo	24.0	22.4	33.4	10.9
Control 4 Hippo	5.6	13.9	7.4	7.2
Control (Path) 3 Hippo	3.9	0.0	6.2	0.0
AD 1 Temporal Ctx	36.7	27.3	60.1	8.9
AD 2 Temporal Ctx	18.3	46.2	39.4	9.0
AD 3 Temporal Ctx	10.1	18.9	10.3	0.0
AD 4 Temporal Ctx	30.4	31.7	52.3	4.0
AD 5 Inf Temporal Ctx	35.2	52.5	85.4	29.9
AD 5 SupTemporal Ctx	10.8	28.7	36.7	11.1
AD 6 Inf Temporal Ctx	27.5	60.7	87.9	31.6
AD 6 Sup Temporal Ctx	22.4	52.6	72.1	17.7
Control 1 Temporal Ctx	3.9	6.0	15.6	0.0
Control 2 Temporal Ctx	7.6	13.0	11.5	3.4
Control 3 Temporal Ctx	9.7	4.4	12.6	0.0
Control 4 Temporal Ctx	11.5	6.8	15.3	0.0
Control (Path) 1 Temporal Ctx	43.7	36.2	67.4	15.7
Control (Path) 2 Temporal Ctx	17.8	22.3	35.6	6.3
Control (Path) 3 Temporal Ctx	1.3	0.0	1.4	0.0
Control (Path) 4 Temporal Ctx	18.2	36.9	35.1	5.9
AD 1 Occipital Ctx	16.3	17.6	35.5	3.5
AD 2 Occipital Ctx (Missing)	0.0	0.0	0.0	0.0
AD 3 Occipital Ctx	10.3	18.9	13.1	0.0
AD 4 Occipital Ctx	16.4	20.0	57.4	3.8
AD 5 Occipital Ctx	11.8	22.3	27.6	4.5
AD 6 Occipital Ctx	3.6	13.5	18.0	6.2
Control 1 Occipital Ctx	7.2	2.6	7.7	0.0
Control 2 Occipital Ctx	0.1	25.3	55.5	12.4
Control 3 Occipital Ctx	19.4	32.4	29.2	10.5
Control 4 Occipital Ctx	10.0	8.2	20.9	6.2
Control (Path) 1 Occipital Ctx	63.1	100.0	100.0	100.0
Control (Path) 2 Occipital Ctx	23.4	12.9	47.7	15.2
Control (Path) 3 Occipital Ctx	1.1	5.2	2.2	0.0

Control (Path) 4 Occipital Ctx	30.8	39.1	45.9	8.0
Control 1 Parietal Ctx	14.3	10.0	35.0	3.7
Control 2 Parietal Ctx	17.4	30.4	61.2	20.2
Control 3 Parietal Ctx	15.5	25.7	26.2	6.3
Control (Path) 1 Parietal Ctx	27.1	64.7	60.3	26.5
Control (Path) 2 Parietal Ctx	56.9	43.8	54.5	17.8
Control (Path) 3 Parietal Ctx	2.0	7.3	0.0	0.0
Control (Path) 4 Parietal Ctx	48.2	82.9	65.6	24.9

Panel 1.2 Summary Ag1501 The GMAC073079_A gene is expressed at moderate levels throughout many of the samples in this panel. Highest expression is detected in an ovarian cancer cell line (CT=30.7). In addition, this gene is overexpressed in all six ovarian cancer cell lines present in this panel when compared to expression in normal ovary. The GMAC073079_A gene is also moderately expressed in cell lines derived from melanoma, breast cancer, and lung cancer. Thus, the expression of this gene could be used to distinguish these cell lines from other tissue samples. In addition, therapeutic modulation of the GMAC073079_A gene or its protein product, through the use of small molecule drugs or antibodies, might be useful in the treatment of ovarian cancer, breast cancer, lung cancer or melanoma.

Among tissues involved in metabolic function, the GMAC073079_A gene is moderately expressed in the adrenal gland, heart, skeletal muscle, and adult liver. Interestingly, GMAC073079_A gene expression is much lower in fetal liver and heart tissues than in the corresponding adult tissues. Thus, expression of the GMAC073079_A gene could be used to differentiate between adult and fetal tissues derived from the heart and liver. Furthermore, this gene or its protein product may be important in the pathogenesis and/or treatment of disease in any or all of the above-named tissues.

There is widespread moderate expression of the GMAC073079_A gene across many of the samples derived from the CNS, including the amygdala, cerebellum, hippocampus, thalamus, cerebral cortex, and spinal cord. Please see CNS_neurodegeneration_panel_v1.0 summary for description of potential utility in the treatment of CNS disorders.

Panel 1.3D Summary Ag2610/Ag2607/Ag1585/Ag2377 Expression of the GMAC073079_A gene appears to be limited to tissues involved in central nervous system function on this panel. Specifically, low but significant expression is detected in the thalamus, substantia nigra, spinal cord and fetal brain. **Ag2545** Expression of the GMAC073079_A gene is low/undetectable (Ct values >35) in all samples on this panel (data not shown).

Panel 2.2 Summary Ag2377 Expression of the GMAC073079_A gene is highest in a sample derived from a breast cancer sample (CT=34.7). Thus, the expression of this gene could be used to distinguish breast cancer samples from other samples and as a diagnostic marker for the presence of breast cancer. Furthermore, therapeutic modulation of the GMAC073079_A gene or the activity of its protein product, through the use of small molecule drugs or antibodies, might be effective in the treatment of breast cancer.

Ag2610/Ag2607/Ag1585 Expression of the GMAC073079_A gene is low/undetectable (Ct values >35) in all samples on this panel (data not shown).

Panel 4D Summary Ag2607/Ag1585/Ag2377 Experiments using three different probe/primer sets show disparate results and are uninterpretable.

Panel CNS_1 Summary Ag2377 Two experiments with the same probe and primer set produce results that are in very good agreement. Expression of the GMAC073079_A gene is highest in the substantia nigra of a Huntington's disease patient, indicating that this gene may participate in the genetic dysregulation associated with the neurodegeneration that occurs in this brain region. The substantia nigra is also critical to the progression of Parkinson's disease neurodegeneration. Thus, pharmacological targeting of the GPCR encoded by the GMAC073079_A gene may help counter this genetic dysregulation and contribute to the restoration of normal function in Huntington's disease as well as potentially Parkinson's disease patients. Pharmacological modulation of GPCR signaling systems is the mechanism by which powerful depression therapies, such as SSRIs, exert their effect.

Panel CNS_1.1 Summary Ag2377 In two experiments using the same probe and primer, highest expression is seen in the cingulate gyrus of patients with para supranuclear palsy PSP (CTs = 32) and depression. This observation indicates that targeting this GPCR could have therapeutic value in the treatment of these diseases.

Panel CNS_neurodegeneration_v1.0 Summary Ag2610/Ag2607/Ag2377/Ag2545 The GMAC073079_A gene is expressed more highly in the temporal cortex of Alzheimer's diseased brain than in control brain without amyloid plaques, which are diagnostic and potentially causative of Alzheimer's disease. The GMAC073079_A gene encodes a protein with homology to GPCRs. GPCRs are readily targetable with drugs, and regulate many specific brain processes, including signaling processes, that are currently the target of FDA-approved pharmaceuticals that treat Alzheimer's disease, such as the cholinergic system.

In Alzheimer's disease abnormal proteolytic processing of amyloid precursor protein (APP) is the central step that leads to formation of amyloid plaque, neurofibrillary tangles, and neuronal loss.

The plaques, which accumulate extracellularly in the brain, are composed of aggregates and cause direct neurotoxic effects and/or increase neuronal vulnerability to excitotoxic insults. The aggregates consist of soluble pathologic amyloid beta peptides AbetaP[1-42] and AbetaP[1-43] and soluble nonpathologic AbetaP[1-40]. Both APP and AbetaP interact with ion transport systems. The major mechanisms proposed for AbetaP-induced cytotoxicity involve the loss of Ca²⁺ homeostasis and the generation of reactive oxygen species (ROS). Kourie, J.I., *Cell Mol Neurobiol* 2001 Jun;21(3):173-213.

The changes in Ca²⁺ homeostasis could be the result of changes in G-protein-driven releases of second messengers. Thus, targeting this class of molecule can have therapeutic potential in Alzheimer's disease treatment. In particular, the increased GMAC073079_A gene expression in brains affected by Alzheimer's indicates potential therapeutic value to drugs that target this GPCR.

GPCR2 (also referred to as AC073079_da2)

Expression of gene AC073079_da2 was assessed using the primer-probe sets Ag2611, Ag2609, and Ag1500, described in Tables 16A and 16B. Results from RTQ-PCR runs are shown in Tables 16C, 16D, 16E, 16F, 16G, and 16H.

Table 16A. Probe Name Ag2611/Ag1500 (identical sequences) (SEQ ID NO: 141, 142, 143)

Primers	Sequences	TM	Length	Start Position
Forward	5'-TGATTGTCTGTGTGGATAAAG-3'	58.5	22	143
Probe	FAM-5'-TCTTCCTCAGCCACCTCTCTACCCTG-3'-TAMRA	68.9	26	185
Reverse	5'-TTATGGTTGTGACCAGGATCTC-3'	58.9	22	211

Table 16B. Probe Name Ag2609 (SEQ ID NO: 144, 145, 146)

Primers	Sequences	TM	Length	Start Position
Forward	5'-CATTGTGATTGTCTGTGTGGAT-3'	58.3	22	138
Probe	FAM-5'-TCTTCCTCAGCCACCTCTCTACCCTG-3'-TAMRA	68.9	26	185
Reverse	5'-TTATGGTTGTGACCAGGATCTC-3'	58.9	22	211

Table 16C. Panel 1.2

Tissue Name	Relative Expression(%) 1.2tm2102f_ag1500	Tissue Name	Relative Expression(%) 1.2tm2102f_ag1500
Endothelial cells	3.1	Renal ca. 786-0	8.0
Heart (fetal)	2.7	Renal ca. A498	11.5

Pancreas	0.0	Renal ca. RXF 393	3.6
Pancreatic ca. CAPAN 2	2.8	Renal ca. ACHN	4.2
Adrenal Gland (new lot*)	8.4	Renal ca. UO-31	8.0
Thyroid	0.0	Renal ca. TK-10	24.5
Salivary gland	39.8	Liver	0.0
Pituitary gland	1.4	Liver (fetal)	1.1
Brain (fetal)	1.2	Liver ca. (hepatoblast) HepG2	1.9
Brain (whole)	5.0	Lung	0.0
Brain (amygdala)	4.3	Lung (fetal)	1.0
Brain (cerebellum)	13.7	Lung ca. (small cell) LX-1	30.4
Brain (hippocampus)	31.4	Lung ca. (small cell) NCI-H69	65.5
Brain (thalamus)	50.7	Lung ca. (s.cell var.) SHP-77	1.5
Cerebral Cortex	100.0	Lung ca. (large cell) NCI-H460	23.2
Spinal cord	18.3	Lung ca. (non-sm. cell) A549	13.9
CNS ca. (glio/astro) U87-MG	12.1	Lung ca. (non-s.cell) NCI-H23	31.4
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca. (non-s.cell) HOP-62	28.5
CNS ca. (astro) SW1783	11.6	Lung ca. (non-s.cl) NCI-H522	51.0
CNS ca.* (neuro; met) SK-N-AS	8.5	Lung ca. (squam.) SW 900	4.3
CNS ca. (astro) SF-539	1.1	Lung ca. (squam.) NCI-H596	22.4
CNS ca. (astro) SNB-75	22.4	Mammary gland	18.2
CNS ca. (glio) SNB-19	19.1	Breast ca.* (pl. effusion) MCF-7	4.4
CNS ca. (glio) U251	2.4	Breast ca.* (pl.ef) MDA-MB-231	0.0
CNS ca. (glio) SF-295	12.2	Breast ca.* (pl. effusion) T47D	91.4
Heart	15.5	Breast ca. BT-549	10.6
Skeletal Muscle (new lot*)	7.0	Breast ca. MDA-N	63.3
Bone marrow	1.7	Ovary	7.9
Thymus	0.9	Ovarian ca. OVCAR-3	8.1
Spleen	0.0	Ovarian ca. OVCAR-4	20.2
Lymph node	0.0	Ovarian ca. OVCAR-5	95.9
Colorectal	6.2	Ovarian ca. OVCAR-8	88.3
Stomach	0.0	Ovarian ca. IGROV-1	24.7
Small intestine	2.4	Ovarian ca.* (ascites) SK-OV-3	16.4
Colon ca. SW480	1.1	Uterus	1.1
Colon ca.* (SW480 met)SW620	5.8	Placenta	27.7
Colon ca. HT29	14.2	Prostate	3.2
Colon ca. HCT-116	3.2	Prostate ca.* (bone met)PC-3	14.5
Colon ca. CaCo-2	10.8	Testis	0.0
83219 CC Well to Mod Diff (ODO3866)	17.8	Melanoma Hs688(A).T	2.3
Colon ca. HCC-2998	6.9	Melanoma* (met) Hs688(B).T	12.3

Gastric ca.* (liver met) NCI-N87	12.2	Melanoma UACC-62	54.7
Bladder	17.4	Melanoma M14	89.5
Trachea	0.0	Melanoma LOX IMVI	0.0
Kidney	14.9	Melanoma* (met) SK-MEL-5	21.5
Kidney (fetal)	16.7		

Table 16D. Panel 1.3D

Tissue Name	Relative Expression(%)	Relative Expression(%)
	1.3dx4tm5657 f ag2609 b2	1.3dx4tm5656 f ag2611 b1
Liver adenocarcinoma	0.0	0.0
Pancreas	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	7.0
Adrenal gland	12.9	0.0
Thyroid	0.0	0.0
Salivary gland	6.2	0.0
Pituitary gland	0.0	0.0
Brain (fetal)	26.4	0.0
Brain (whole)	26.2	26.8
Brain (amygdala)	3.9	5.4
Brain (cerebellum)	0.0	6.5
Brain (hippocampus)	17.1	6.2
Brain (substantia nigra)	31.8	78.0
Brain (thalamus)	82.3	100.0
Cerebral Cortex	0.0	19.1
Spinal cord	100.0	99.4
CNS ca. (glio/astro) U87-MG	0.0	0.0
CNS ca. (glio/astro) U-118-MG	0.0	0.0
CNS ca. (astro) SW1783	6.9	0.0
CNS ca.* (neuro; met) SK-N-AS	0.0	0.0
CNS ca. (astro) SF-539	0.0	0.0
CNS ca. (astro) SNB-75	0.0	0.0
CNS ca. (glio) SNB-19	5.7	5.7
CNS ca. (glio) U251	0.0	0.0
CNS ca. (glio) SF-295	6.7	0.0
Heart (fetal)	0.0	6.1
Heart	0.0	0.0
Fetal Skeletal	0.0	0.0
Skeletal muscle	0.0	0.0

Bone marrow	0.0	0.0
Thymus	0.0	0.0
Spleen	0.0	0.0
Lymph node	0.0	0.0
Colorectal	6.7	0.0
Stomach	0.0	0.0
Small intestine	0.0	15.3
Colon ca. SW480	0.0	0.0
Colon ca.* (SW480 met)SW620	0.0	0.0
Colon ca. HT29	0.0	0.0
Colon ca. HCT-116	0.0	0.0
Colon ca. CaCo-2	0.0	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	0.0
Colon ca. HCC-2998	0.0	0.0
Gastric ca.* (liver met) NCI-N87	0.0	0.0
Bladder	0.0	0.0
Trachea	0.0	0.0
Kidney	0.0	0.0
Kidney (fetal)	0.0	0.0
Renal ca. 786-0	0.0	0.0
Renal ca. A498	0.0	0.0
Renal ca. RXF 393	12.7	16.4
Renal ca. ACHN	0.0	0.0
Renal ca. UO-31	0.0	0.0
Renal ca. TK-10	7.4	0.0
Liver	0.0	0.0
Liver (fetal)	0.0	0.0
Liver ca. (hepatoblast) HepG2	0.0	8.0
Lung	0.0	0.0
Lung (fetal)	0.0	0.0
Lung ca. (small cell) LX-1	19.3	2.6
Lung ca. (small cell) NCI-H69	0.0	0.0
Lung ca. (s.cell var.) SHP-77	0.0	0.0
Lung ca. (large cell)NCI-H460	0.0	0.0
Lung ca. (non-sm. cell) A549	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	10.2	0.0
Lung ca (non-s.cell) HOP-62	0.0	6.2
Lung ca. (non-s.cl) NCI-H522	0.0	0.0
Lung ca. (squam.) SW 900	0.0	0.0
Lung ca. (squam.) NCI-H596	0.0	0.0
Mammary gland	0.0	0.0
Breast ca.* (pl. effusion) MCF-7	0.0	0.0

Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0
Breast ca.* (pl. effusion) T47D	22.1	32.4
Breast ca. BT-549	0.0	0.0
Breast ca. MDA-N	3.2	29.7
Ovary	0.0	0.0
Ovarian ca. OVCAR-3	0.0	0.0
Ovarian ca. OVCAR-4	0.0	0.0
Ovarian ca. OVCAR-5	10.6	0.0
Ovarian ca. OVCAR-8	6.0	32.6
Ovarian ca. IGROV-1	0.0	0.0
Ovarian ca.* (ascites) SK-OV-3	8.8	0.0
Uterus	0.0	0.0
Placenta	38.7	27.9
Prostate	0.0	0.0
Prostate ca.* (bone met)PC-3	0.0	6.4
Testis	34.9	14.8
Melanoma Hs688(A).T	0.0	0.0
Melanoma* (met) Hs688(B).T	0.0	0.0
Melanoma UACC-62	0.0	32.6
Melanoma M14	0.0	18.4
Melanoma LOX IMVI	0.0	0.0
Melanoma* (met) SK-MEL-5	7.9	0.0
Adipose	0.0	0.0

Table 16E. Panel 2.2

Tissue Name	Relative Expression(%)	Relative Expression(%)
	2.2x4tm6501 f_ag2609_a1	2.2x4tm6501 f_ag2611_a2
Normal Colon GENPAK 061003	6.3	0.0
97759 Colon cancer (OD06064)	0.0	0.0
97760 Colon cancer NAT (OD06064)	0.0	0.0
97778 Colon cancer (OD06159)	0.0	0.0
97779 Colon cancer NAT (OD06159)	5.4	0.0
98861 Colon cancer (OD06297-04)	0.0	0.0
98862 Colon cancer NAT (OD06297-015)	6.1	0.0
83237 CC Gr.2 ascend colon (ODO3921)	0.0	0.0
83238 CC NAT (ODO3921)	0.0	0.0
97766 Colon cancer metastasis (OD06104)	0.0	0.0
97767 Lung NAT (OD06104)	0.0	0.0
87472 Colon mets to lung (OD04451-01)	0.0	0.0

87473 Lung NAT (OD04451-02)	0.0	0.0
Normal Prostate Clontech A+ 6546-1 (8090438)	0.0	0.0
84140 Prostate Cancer (OD04410)	0.0	0.0
84141 Prostate NAT (OD04410)	0.0	0.0
Normal Ovary Res. Gen.	0.0	0.0
98863 Ovarian cancer (OD06283-03)	0.0	0.0
98865 Ovarian cancer NAT/fallopian tube (OD06283-07)	7.1	10.9
Ovarian Cancer GENPAK 064008	28.8	0.0
97773 Ovarian cancer (OD06145)	0.0	5.6
97775 Ovarian cancer NAT (OD06145)	15.9	0.0
98853 Ovarian cancer (OD06455-03)	6.8	0.0
98854 Ovarian NAT (OD06455-07) Fallopian tube	0.0	0.0
Normal Lung GENPAK 061010	0.0	0.0
92337 Invasive poor diff. lung adeno (ODO4945-01)	0.0	0.0
92338 Lung NAT (ODO4945-03)	3.1	0.0
84136 Lung Malignant Cancer (OD03126)	0.0	0.0
84137 Lung NAT (OD03126)	0.0	0.0
90372 Lung Cancer (OD05014A)	0.0	0.0
90373 Lung NAT (OD05014B)	0.0	4.3
97761 Lung cancer (OD06081)	0.0	0.0
97762 Lung cancer NAT (OD06081)	0.0	8.0
85950 Lung Cancer (OD04237-01)	0.0	0.0
85970 Lung NAT (OD04237-02)	0.0	0.0
83255 Ocular Mel Met to Liver (ODO4310)	0.0	10.3
83256 Liver NAT (ODO4310)	0.0	0.0
84139 Melanoma Mets to Lung (OD04321)	16.5	15.2
84138 Lung NAT (OD04321)	0.0	0.0
Normal Kidney GENPAK 061008	7.4	11.0
83786 Kidney Ca, Nuclear grade 2 (OD04338)	4.3	0.0
83787 Kidney NAT (OD04338)	0.0	2.8
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	5.6	10.1
83789 Kidney NAT (OD04339)	0.0	7.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	7.3
83791 Kidney NAT (OD04340)	0.0	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0
83793 Kidney NAT (OD04348)	14.6	12.7
98938 Kidney malignant cancer (OD06204B)	9.8	9.6
98939 Kidney normal adjacent tissue (OD06204E)	0.0	0.0
85973 Kidney Cancer (OD04450-01)	18.0	0.0
85974 Kidney NAT (OD04450-03)	0.0	0.0
Kidney Cancer Clontech 8120613	0.0	0.0
Kidney NAT Clontech 8120614	0.0	0.0

Kidney Cancer Clontech 9010320	0.0	0.0
Kidney NAT Clontech 9010321	0.0	0.0
Kidney Cancer Clontech 8120607	0.0	0.0
Kidney NAT Clontech 8120608	0.0	0.0
Normal Uterus GENPAK 061018	9.8	14.4
Uterus Cancer GENPAK 064011	0.0	0.0
Normal Thyroid Clontech A+ 6570-1 (7080817)	0.0	0.0
Thyroid Cancer GENPAK 064010	0.0	0.0
Thyroid Cancer INVITROGEN A302152	0.0	15.1
Thyroid NAT INVITROGEN A302153	0.0	0.0
Normal Breast GENPAK 061019	18.7	33.9
84877 Breast Cancer (OD04566)	0.0	0.0
Breast Cancer Res. Gen. 1024	21.1	17.7
85975 Breast Cancer (OD04590-01)	0.0	0.0
85976 Breast Cancer Mets (OD04590-03)	8.4	0.0
87070 Breast Cancer Metastasis (OD04655-05)	0.0	0.0
GENPAK Breast Cancer 064006	1.5	9.9
Breast Cancer Clontech 9100266	100.0	100.0
Breast NAT Clontech 9100265	5.9	7.8
Breast Cancer INVITROGEN A209073	3.7	7.0
Breast NAT INVITROGEN A2090734	0.0	27.6
97763 Breast cancer (OD06083)	9.9	14.2
97764 Breast cancer node metastasis (OD06083)	13.5	5.3
Normal Liver GENPAK 061009	0.0	0.0
Liver Cancer Research Genetics RNA 1026	0.0	0.0
Liver Cancer Research Genetics RNA 1025	0.0	0.0
Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.0	0.0
Paired Liver Tissue Research Genetics RNA 6004-N	0.0	0.0
Paired Liver Cancer Tissue Research Genetics RNA 6005-T	5.2	0.0
Paired Liver Tissue Research Genetics RNA 6005-N	0.0	0.0
Liver Cancer GENPAK 064003	0.0	0.0
Normal Bladder GENPAK 061001	0.0	0.0
Bladder Cancer Research Genetics RNA 1023	0.0	9.0
Bladder Cancer INVITROGEN A302173	10.2	0.0
Normal Stomach GENPAK 061017	12.3	0.0
Gastric Cancer Clontech 9060397	0.0	0.0
NAT Stomach Clontech 9060396	0.0	0.0
Gastric Cancer Clontech 9060395	11.4	0.0
NAT Stomach Clontech 9060394	0.0	0.0
Gastric Cancer GENPAK 064005	0.0	0.0

Table 16F. Panel 4D

Tissue Name	Relative Expression(%)		Relative Expression (%)
	4dx4tm4979 f ag2609 b2	4Dx4tm4979 f ag2609 b2	4dx4tm5038 f ag2611 a2
93768_Secondary Th1_anti-CD28/anti-CD3	6.4	6.4	4.8
93769_Secondary Th2_anti-CD28/anti-CD3	12.7	12.7	13.9
93770_Secondary Tr1_anti-CD28/anti-CD3	0.9	0.9	14.7
93573_Secondary Th1_resting day 4-6 in IL-2	3.9	3.9	0.0
93572_Secondary Th2_resting day 4-6 in IL-2	0.0	0.0	0.0
93571_Secondary Tr1_resting day 4-6 in IL-2	0.4	0.4	0.0
93568_primary Th1_anti-CD28/anti-CD3	0.0	0.0	19.5
93569_primary Th2_anti-CD28/anti-CD3	9.2	9.2	0.0
93570_primary Tr1_anti-CD28/anti-CD3	4.1	4.1	7.1
93565_primary Th1_resting dy 4-6 in IL-2	16.8	16.8	20.4
93566_primary Th2_resting dy 4-6 in IL-2	13.6	13.6	14.9
93567_primary Tr1_resting dy 4-6 in IL-2	1.5	1.5	0.0
93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	0.0	0.0	13.4
93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	0.2	0.2	1.3
93251_CD8 Lymphocytes_anti-CD28/anti-CD3	0.0	0.0	4.5
93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	13.5	13.5	4.4
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	13.5	13.5	7.3
93354_CD4_none	10.2	10.2	10.5
93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	16.3	16.3	5.7
93103_LAK cells_resting	17.1	17.1	6.3
93788_LAK cells_IL-2	0.0	0.0	0.0
93787_LAK cells_IL-2+IL-12	2.9	2.9	19.4
93789_LAK cells_IL-2+IFN gamma	7.7	7.7	0.0
93790_LAK cells_IL-2+ IL-18	4.1	4.1	0.0
93104_LAK cells_PMA/ionomycin and IL-18	2.0	2.0	14.1
93578_NK Cells IL-2_resting	18.4	18.4	0.0
93109_Mixed Lymphocyte Reaction_Two Way MLR	15.6	15.6	14.9
93110_Mixed Lymphocyte Reaction_Two Way MLR	15.8	15.8	0.0
93111_Mixed Lymphocyte Reaction_Two Way MLR	0.0	0.0	8.1
93112_Mononuclear Cells (PBMCs)_resting	1.6	1.6	0.0
93113_Mononuclear Cells (PBMCs)_PWM	10.1	10.1	14.1
93114_Mononuclear Cells (PBMCs)_PHA-L	6.7	6.7	20.8
93249_Ramos (B cell)_none	17.1	17.1	7.8
93250_Ramos (B cell)_ionomycin	8.2	8.2	0.0

93349 B lymphocytes PWM	3.7	3.7	4.4
93350 B lymphocytes CD40L and IL-4	7.2	7.2	13.2
92665 EOL-1 (Eosinophil) dbcAMP differentiated	9.4	9.4	2.1
93248 EOL-1 (Eosinophil) dbcAMP/PMA/ionomycin	25.0	25.0	0.0
93356 Dendritic Cells none	43.3	43.3	27.2
93355 Dendritic Cells LPS 100 ng/ml	17.5	17.5	11.3
93775 Dendritic Cells anti-CD40	24.1	24.1	5.5
93774 Monocytes resting	2.2	2.2	0.0
93776 Monocytes LPS 50 ng/ml	69.1	69.1	100.0
93581 Macrophages resting	100.0	100.0	97.3
93582 Macrophages LPS 100 ng/ml	14.9	14.9	8.1
93098 HUVEC (Endothelial) none	0.0	0.0	0.0
93099 HUVEC (Endothelial) starved	4.7	4.7	19.0
93100 HUVEC (Endothelial) IL-1b	0.0	0.0	0.0
93779 HUVEC (Endothelial) IFN gamma	4.9	4.9	0.0
93102 HUVEC (Endothelial) TNF alpha + IFN gamma	15.3	15.3	6.9
93101 HUVEC (Endothelial) TNF alpha + IL4	18.5	18.5	0.0
93781 HUVEC (Endothelial) IL-11	0.8	0.8	6.0
93583 Lung Microvascular Endothelial Cells none	6.5	6.5	65.9
93584 Lung Microvascular Endothelial Cells TNFa (4 ng/ml) and IL1b (1 ng/ml)	9.5	9.5	18.3
92662 Microvascular Dermal endothelium none	0.4	0.4	0.0
92663 Microvascular Dermal endothelium TNFa (4 ng/ml) and IL1b (1 ng/ml)	16.1	16.1	3.0
93773 Bronchial epithelium TNFa (4 ng/ml) and IL1b (1 ng/ml) **	1.3	1.3	6.3
93347 Small Airway Epithelium none	2.9	2.9	0.0
93348 Small Airway Epithelium TNFa (4 ng/ml) and IL1b (1 ng/ml)	10.9	10.9	3.9
92668 Coronary Artery SMC resting	0.0	0.0	0.0
92669 Coronary Artery SMC TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.4	0.4	5.9
93107 astrocytes resting	9.9	9.9	0.0
93108 astrocytes TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	0.0
92666 KU-812 (Basophil) resting	3.1	3.1	4.0
92667 KU-812 (Basophil) PMA/ionomycin	3.9	3.9	4.8
93579 CCD1106 (Keratinocytes) none	9.6	9.6	0.0
93580 CCD1106 (Keratinocytes) TNFa and IFNg **	6.5	6.5	0.0
93791 Liver Cirrhosis	22.6	22.6	25.9
93792 Lupus Kidney	3.0	3.0	0.0
93577 NCI-H292	4.4	4.4	0.0
93358 NCI-H292 IL-4	3.5	3.5	0.0
93360 NCI-H292 IL-9	1.5	1.5	0.0

93359_NCI-H292_IL-13	9.5	9.5	0.0
93357_NCI-H292_IFN gamma	0.0	0.0	0.0
93777_HPAEC_-	0.0	0.0	0.0
93778_HPAEC_IL-1 beta/TNA alpha	3.2	3.2	0.0
93254_Normal Human Lung Fibroblast_none	4.1	4.1	0.0
93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0	0.0	0.0
93257_Normal Human Lung Fibroblast_IL-4	5.7	5.7	0.0
93256_Normal Human Lung Fibroblast_IL-9	0.0	0.0	0.0
93255_Normal Human Lung Fibroblast_IL-13	0.0	0.0	0.0
93258_Normal Human Lung Fibroblast_IFN gamma	0.0	0.0	0.0
93106_Dermal Fibroblasts CCD1070_resting	5.0	5.0	13.6
93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	14.7	14.7	36.0
93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	0.0	0.0	6.3
93772_dermal fibroblast_IFN gamma	0.0	0.0	0.0
93771_dermal fibroblast_IL-4	0.0	0.0	6.3
93260_IBD Colitis 2	9.3	9.3	0.0
93261_IBD Crohns	0.0	0.0	0.0
735010_Colon normal	1.0	1.0	6.8
735019_Lung_none	11.7	11.7	19.7
64028-1_Thymus_none	19.4	19.4	20.1
64030-1_Kidney_none	4.4	4.4	20.1

Table 16G. Panel CNS_1

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	CNS1x4tm6189 f_ag2609_a2		CNS1x4tm6189 f_ag2609_a2
102633_BA4 Control	0.0	102605_BA17 PSP	7.2
102641_BA4 Control2	6.4	102612_BA17 PSP2	0.0
102625_BA4 Alzheimer's2	6.6	102637_Sub Nigra Control	49.2
102649_BA4 Parkinson's	7.2	102645_Sub Nigra Control2	37.0
102656_BA4 Parkinson's2	0.0	102629_Sub Nigra Alzheimer's2	6.5
102664_BA4 Huntington's	14.6	102660_Sub Nigra Parkinson's2	19.1
102671_BA4 Huntington's2	0.0	102667_Sub Nigra Huntington's	100.0
102603_BA4 PSP	6.0	102674_Sub Nigra Huntington's2	7.0

102610_BA4 PSP2	8.4	102614_Sub Nigra PSP2	4.5
102588_BA4 Depression	12.2	102592_Sub Nigra Depression	0.0
102596_BA4 Depression2	5.1	102599_Sub Nigra Depression2	4.8
102634_BA7 Control	18.5	102636_Glob Palladus Control	9.4
102642_BA7 Control2	0.0	102644_Glob Palladus Control2	6.4
102626_BA7 Alzheimer's2	5.6	102620_Glob Palladus Alzheimer's	4.8
102650_BA7 Parkinson's	3.7	102628_Glob Palladus Alzheimer's2	13.5
102657_BA7 Parkinson's2	0.0	102652_Glob Palladus Parkinson's	38.1
102665_BA7 Huntington's	8.8	102659_Glob Palladus Parkinson's2	11.9
102672_BA7 Huntington's2	9.5	102606_Glob Palladus PSP	0.0
102604_BA7 PSP	14.9	102613_Glob Palladus PSP2	0.0
102611_BA7 PSP2	0.0	102591_Glob Palladus Depression	23.4
102589_BA7 Depression	9.1	102638_Temp Pole Control	0.0
102632_BA9 Control	5.9	102646_Temp Pole Control2	0.0
102640_BA9 Control2	10.5	102622_Temp Pole Alzheimer's	0.0
102617_BA9 Alzheimer's	0.0	102630_Temp Pole Alzheimer's2	0.0
102624_BA9 Alzheimer's2	0.0	102653_Temp Pole Parkinson's	0.0
102648_BA9 Parkinson's	18.7	102661_Temp Pole Parkinson's2	3.2
102655_BA9 Parkinson's2	5.4	102668_Temp Pole Huntington's	0.0
102663_BA9 Huntington's	11.6	102607_Temp Pole PSP	0.0
102670_BA9 Huntington's2	0.0	102615_Temp Pole PSP2	0.0
102602_BA9 PSP	3.3	102600_Temp Pole Depression2	0.0
102609_BA9 PSP2	0.0	102639_Cing Gyr Control	17.9
102587_BA9 Depression	4.8	102647_Cing Gyr Control2	9.3
102595_BA9 Depression2	0.0	102623_Cing Gyr Alzheimer's	11.9
102635_BA17 Control	9.6	102631_Cing Gyr Alzheimer's2	0.0
102643_BA17 Control2	8.4	102654_Cing Gyr Parkinson's	38.3
102627_BA17 Alzheimer's2	0.0	102662_Cing Gyr	11.8

		Parkinson's2	
102651_BA17 Parkinson's	18.4	102669_Cing Gyr Huntington's	28.9
102658_BA17 Parkinson's2	20.4	102676_Cing Gyr Huntington's2	25.4
102666_BA17 Huntington's	17.8	102608_Cing Gyr PSP	33.7
102673_BA17 Huntington's2	0.0	102616_Cing Gyr PSP2	13.7
102590_BA17 Depression	12.1	102594_Cing Gyr Depression	17.8
102597_BA17 Depression2	8.5	102601_Cing Gyr Depression2	19.6

Table 16H. Panel CNS_neurodegeneration_v1.0

Tissue Name	Relative Expression(%)	Relative Expression(%)
	tm7047f_ag2609_b1_s1	tm7047f_ag2611_b1_s2
AD 1 Hippo	12.6	2.2
AD 2 Hippo	39.4	11.1
AD 3 Hippo	7.3	0.9
AD 4 Hippo	4.2	0.8
AD 5 Hippo	36.3	14.0
AD 6 Hippo	63.9	11.3
Control 2 Hippo	11.2	3.5
Control 4 Hippo	2.5	1.0
Control (Path) 3 Hippo	5.0	1.1
AD 1 Temporal Ctx	21.3	7.6
AD 2 Temporal Ctx	31.3	12.1
AD 3 Temporal Ctx	9.5	1.9
AD 4 Temporal Ctx	28.1	3.7
AD 5 Inf Temporal Ctx	74.9	13.6
AD 5 Sup Temporal Ctx	18.2	4.3
AD 6 Inf Temporal Ctx	54.8	16.2
AD 6 Sup Temporal Ctx	39.7	13.6
Control 1 Temporal Ctx	3.2	1.0
Control 2 Temporal Ctx	7.1	3.1
Control 3 Temporal Ctx	8.2	2.7
Control 3 Temporal Ctx	6.2	5.1
Control (Path) 1 Temporal Ctx	34.6	9.7
Control (Path) 2 Temporal Ctx	19.6	5.2
Control (Path) 3 Temporal Ctx	0.0	0.8
Control (Path) 4 Temporal Ctx	12.7	2.5

AD 1 Occipital Ctx	12.7	100.0
AD 2 Occipital Ctx (Missing)	0.0	0.0
AD 3 Occipital Ctx	6.2	1.8
AD 4 Occipital Ctx	9.7	3.9
AD 5 Occipital Ctx	13.3	4.7
AD 6 Occipital Ctx	19.9	4.1
Control 1 Occipital Ctx	16.0	2.0
Control 2 Occipital Ctx	24.6	7.1
Control 3 Occipital Ctx	8.8	4.5
Control 4 Occipital Ctx	4.6	2.9
Control (Path) 1 Occipital Ctx	100.0	17.9
Control (Path) 2 Occipital Ctx	17.9	5.8
Control (Path) 3 Occipital Ctx	2.8	1.8
Control (Path) 4 Occipital Ctx	12.3	6.7
Control 1 Parietal Ctx	20.4	8.1
Control 2 Parietal Ctx	19.3	4.9
Control 3 Parietal Ctx	30.2	6.8
Control (Path) 1 Parietal Ctx	40.8	11.5
Control (Path) 2 Parietal Ctx	21.1	6.3
Control (Path) 3 Parietal Ctx	3.7	0.7
Control (Path) 4 Parietal Ctx	32.0	8.6

Panel 1.2 Summary Ag1500 Highest expression of the AC073079_da2 gene is seen in the cerebral cortex (CT=30.4). Among tissues active in the central nervous system, the AC073079_da2 gene is also moderately expressed in the cerebellum, hippocampus, thalamus and spinal cord. Please see CNS_neurodegeneration_panel_v1.0 summary for description of the potential utility of this gene in the treatment of CNS diseases.

Among tissues with metabolic function, the AC073079_da2 gene is expressed at low but significant levels in samples derived from the adrenal gland, heart and skeletal muscle. Therefore, the protein encoded by the AC073079_da2 gene may be important in the pathogenesis and/or treatment of disease in any or all of the above-named tissues.

The AC073079_da2 gene also shows an association with cancerous cell lines and is expressed in clusters of samples derived from breast, ovarian, melanoma and lung cancer cell lines. Thus, the expression of this gene could be used to distinguish samples derived from cell lines when compared to tissues. In addition, therapeutic modulation of the AC073079_da2 gene or its protein product, through the use of small molecule drugs or antibodies, might be beneficial in the treatment of ovarian cancer, breast cancer, lung cancer or melanoma.

Panel 1.3D Summary Ag2611/Ag2609 Two experiments with two different probe/primer sets both show preferential expression of the AC073079_da2 gene in tissues originating in the central nervous system, with expression seen in the spinal cord (CT=33.1) and thalamus (CT=34.1). Please see CNS_neurodegeneration_panel_v1.0 summary for
5 description of the potential utility of this gene in the treatment of CNS diseases.

Panel 2.2 Summary Ag2611/Ag2609 In two experiments using two different probe and primer sets, expression of the AC073079_da2 gene is limited to a sample derived from a breast cancer (CT=33.2) and appears to be overexpressed in breast cancer as compared to normal adjacent tissue. This suggests that the AC073079_da2 gene could be used to distinguish breast cancer samples from
10 other samples and for the detection of breast cancer. Moreover, therapeutic inhibition of this gene, through the use of small molecule drugs or antibodies might be of use in the treatment of breast cancer.

Panel 4D Summary Ag2611/Ag2609 The AC073079_da2 gene is expressed at moderate levels in LPS-activated monocytes but not in resting monocytes. Conversely, the AC073079_da2 gene is expressed at moderate levels in resting macrophages, but at low levels
15 in activated macrophages. This pattern is evident in experiments using two different probe and primer sets that match the AC073079_da2 sequence. Since circulating monocytes and tissue macrophages are both developmentally related cell types, the AC073079_da2 gene could serve as a useful target for the development of small molecule drugs as well as therapeutic antibodies. Therapeutic antibodies and small molecule inhibitors that block the function of the
20 protein encoded by the AC073079_da2 gene may be useful in reducing inflammation and autoimmune disease symptoms in patients with Crohn's disease, inflammatory bowel disease, asthma, psoriasis, and rheumatoid arthritis.

Panel CNS_1 Summary Ag2609 Expression of the AC073079_da2 gene is highest in the substantia nigra of a Huntington's disease patient, indicating that this gene may participate
25 in the genetic dysregulation associated with the neurodegeneration that occurs in this brain region. The substantia nigra is also critical to the progression of Parkinson's disease neurodegeneration. Thus, pharmacological targeting of the GPCR encoded by the AC073079_da2 gene may help counter this genetic dysregulation and contribute to the restoration of normal function in Huntington's disease as well as potentially Parkinson's
30 disease patients. Pharmacological modulation of GPCR signaling systems is the mechanism by which powerful depression therapies, such as SSRIs, exert their effect.

Panel CNS_neurodegeneration_v1.0 Summary Ag2611/Ag2609 The

AC073079_da2 gene is expressed more highly in the temporal cortex of Alzheimer's diseased brain than in control brain without amyloid plaques, which are diagnostic and potentially causative of Alzheimer's disease. The AC073079_da2 gene encodes a protein with homology to GPCRs. GPCRs are readily targetable with drugs, and regulate many specific brain processes, including signaling processes, that are currently the target of FDA-approved pharmaceuticals that treat Alzheimer's disease, such as the cholinergic system.

The major mechanisms proposed for AbetaP-induced cytotoxicity involve the loss of Ca²⁺ homeostasis and the generation of reactive oxygen species (ROS). The changes in Ca²⁺ homeostasis could be the result of changes in G-protein-driven releases of second messengers. Thus, targeting this class of molecule can have therapeutic potential in Alzheimer's disease treatment. In particular, the increased AC073079_da2 gene expression in brains affected by Alzheimer's indicates potential therapeutic value to drugs that target this GPCR.

GPCR3 (also referred to as sggc_draft_ba656o22_20000731_da4/ CG55881-02)

Expression of gene sggc_draft_ba656o22_20000731_da4 was assessed using the primer-probe sets Ag1898 and Ag1523, described in Tables 17A and 17B. Please note that Ag1523 contains a single mismatch in the probe relative to the sggc_draft_ba656o22_20000731_da4 sequence. This mismatch is not predicted to alter the RTQ-PCR results. Results from RTQ-PCR runs are shown in Tables 17C, 17D, and 17E.

Table 17A Probe Name Ag1898 (SEQ ID NO: 147, 148, 149)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GGCTGTGGTGTCTCTGTTTAC-3'	58.8	22	740
Probe	TET-5'-CATCTTCATGTATCTCCAGCCAGCCA-3'-TAMRA	69.3	26	770
Reverse	5'-CTATGAACCTGCCCTGCTCAT-3'	59.3	21	808

Table 17B Probe Name Ag1523 (SEQ ID NO: 150, 151, 152)

Primers	Sequences	TM	Length	Start Position
Forward	5'-AGGGCAAGTTCATAGCTCTGTT-3'	59.4	22	809
Probe	TET-5'-CTACACCGTAGTCACTCCTGCGCTGA-3'-TAMRA	69.6	26	831
Reverse	5'-CGTGTTCCTCAGGGTGTAATA-3'	59	22	864

Table 17C. Panel 1.2

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	1.2tm2157t_ag1523		1.2tm2157t_ag1523
Endothelial cells	0.0	Renal ca. 786-0	0.0
Heart (fetal)	0.0	Renal ca. A498	0.3
Pancreas	0.2	Renal ca. RXF 393	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. ACHN	0.0
Adrenal Gland (new lot*)	0.2	Renal ca. UO-31	0.3
Thyroid	0.0	Renal ca. TK-10	0.2
Salivary gland	0.2	Liver	0.0
Pituitary gland	0.0	Liver (fetal)	0.0
Brain (fetal)	0.0	Liver ca. (hepatoblast) HepG2	0.0
Brain (whole)	0.0	Lung	0.0
Brain (amygdala)	0.1	Lung (fetal)	0.0
Brain (cerebellum)	2.0	Lung ca. (small cell) LX-1	0.1
Brain (hippocampus)	0.1	Lung ca. (small cell) NCI-H69	2.4
Brain (thalamus)	0.2	Lung ca. (s.cell var.) SHP-77	0.0
Cerebral Cortex	0.0	Lung ca. (large cell) NCI-H460	0.6
Spinal cord	0.0	Lung ca. (non-sm. cell) A549	1.0
CNS ca. (glio/astro) U87-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca. (non-s.cell) HOP-62	0.6
CNS ca. (astro) SW1783	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
CNS ca.* (neuro; met) SK-N-AS	0.0	Lung ca. (squam.) SW 900	0.2
CNS ca. (astro) SF-539	0.0	Lung ca. (squam.) NCI-H596	1.0
CNS ca. (astro) SNB-75	0.2	Mammary gland	0.0
CNS ca. (glio) SNB-19	0.4	Breast ca.* (pl. effusion) MCF-7	0.0
CNS ca. (glio) U251	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
CNS ca. (glio) SF-295	0.0	Breast ca.* (pl. effusion) T47D	0.6
Heart	0.3	Breast ca. BT-549	0.0
Skeletal Muscle (new lot*)	0.2	Breast ca. MDA-N	10.4
Bone marrow	0.2	Ovary	0.0
Thymus	0.0	Ovarian ca. OVCAR-3	0.0

Spleen	0.0	Ovarian ca. OVCAR-4	0.0
Lymph node	0.0	Ovarian ca. OVCAR-5	1.1
Colorectal	0.2	Ovarian ca. OVCAR-8	0.3
Stomach	0.0	Ovarian ca. IGROV-1	0.4
Small intestine	0.0	Ovarian ca.* (ascites) SK-OV-3	0.4
Colon ca. SW480	0.0	Uterus	0.0
Colon ca.* (SW480 met)SW620	0.0	Placenta	0.1
Colon ca. HT29	0.2	Prostate	19.3
Colon ca. HCT-116	0.0	Prostate ca.* (bone met)PC-3	0.3
Colon ca. CaCo-2	0.0	Testis	2.3
83219 CC Well to Mod Diff (ODO3866)	0.8	Melanoma Hs688(A).T	0.0
Colon ca. HCC-2998	0.0	Melanoma* (met) Hs688(B).T	0.4
Gastric ca.* (liver met) NCI-N87	0.0	Melanoma UACC-62	0.6
Bladder	0.5	Melanoma M14	4.1
Trachea	0.0	Melanoma LOX IMVI	0.2
Kidney	0.0	Melanoma* (met) SK-MEL-5	100.0
Kidney (fetal)	0.2		

Table 17D. Panel 1.3D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	1.3dx4tm5422 t_ag1898_a2		1.3dx4tm5422 t_ag1898_a2
Liver adenocarcinoma	0.0	Kidney (fetal)	0.0
Pancreas	0.0	Renal ca. 786-0	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. A498	0.0
Adrenal gland	0.0	Renal ca. RXF 393	0.0
Thyroid	0.0	Renal ca. ACHN	0.0
Salivary gland	0.0	Renal ca. UO-31	0.0
Pituitary gland	0.0	Renal ca. TK-10	0.0
Brain (fetal)	0.0	Liver	0.0
Brain (whole)	1.7	Liver (fetal)	0.0
Brain (amygdala)	0.0	Liver ca. (hepatoblast) HepG2	0.0
Brain (cerebellum)	29.9	Lung	0.0
Brain (hippocampus)	0.0	Lung (fetal)	0.0
Brain (substantia nigra)	1.2	Lung ca. (small cell) LX-1	0.0
Brain (thalamus)	0.0	Lung ca. (small cell) NCI-	0.0

		H69	
Cerebral Cortex	0.0	Lung ca. (s.cell var.) SHP-77	0.3
Spinal cord	0.0	Lung ca. (large cell)NCI-H460	0.0
CNS ca. (glio/astro) U87-MG	0.0	Lung ca. (non-sm. cell) A549	0.0
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
CNS ca. (astro) SW1783	0.0	Lung ca (non-s.cell) HOP-62	0.0
CNS ca.* (neuro; met) SK-N-AS	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
CNS ca. (astro) SF-539	2.6	Lung ca. (squam.) SW 900	0.0
CNS ca. (astro) SNB-75	0.0	Lung ca. (squam.) NCI-H596	0.0
CNS ca. (glio) SNB-19	0.0	Mammary gland	0.0
CNS ca. (glio) U251	0.0	Breast ca.* (pl. effusion) MCF-7	0.0
CNS ca. (glio) SF-295	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
Heart (fetal)	0.0	Breast ca.* (pl. effusion) T47D	0.0
Heart	0.0	Breast ca. BT-549	0.0
Fetal Skeletal	0.0	Breast ca. MDA-N	4.1
Skeletal muscle	0.0	Ovary	0.8
Bone marrow	0.0	Ovarian ca. OVCAR-3	0.0
Thymus	1.3	Ovarian ca. OVCAR-4	0.0
Spleen	0.0	Ovarian ca. OVCAR-5	0.0
Lymph node	2.2	Ovarian ca. OVCAR-8	0.0
Colorectal	0.0	Ovarian ca. IGROV-1	0.0
Stomach	0.0	Ovarian ca.* (ascites) SK-OV-3	0.0
Small intestine	0.0	Uterus	0.0
Colon ca. SW480	0.0	Placenta	0.0
Colon ca.* (SW480 met)SW620	0.0	Prostate	4.2
Colon ca. HT29	0.0	Prostate ca.* (bone met)PC-3	0.0
Colon ca. HCT-116	0.0	Testis	31.9
Colon ca. CaCo-2	0.0	Melanoma Hs688(A).T	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	Melanoma* (met) Hs688(B).T	0.0
Colon ca. HCC-2998	0.0	Melanoma UACC-62	2.3
Gastric ca.* (liver met) NCI-N87	0.0	Melanoma M14	6.0
Bladder	0.0	Melanoma LOX IMVI	0.0

Trachea	0.0	Melanoma* (met) SK-MEL-5	100.0
Kidney	0.0	Adipose	0.0

Table 17E. Panel 2D

Tissue Name	Relative Expression(%)	
	2Dtm2354t_ ag1523	2dtm2449t_ ag1523
Normal Colon GENPAK 061003	5.2	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	3.7
83220 CC NAT (ODO3866)	3.3	0.0
83221 CC Gr.2 rectosigmoid (ODO3868)	0.0	0.0
83222 CC NAT (ODO3868)	0.0	4.2
83235 CC Mod Diff (ODO3920)	0.0	0.0
83236 CC NAT (ODO3920)	0.0	0.0
83237 CC Gr.2 ascend colon (ODO3921)	2.0	0.0
83238 CC NAT (ODO3921)	0.0	0.0
83241 CC from Partial Hepatectomy (ODO4309)	0.0	0.0
83242 Liver NAT (ODO4309)	0.0	2.0
87472 Colon mets to lung (OD04451-01)	0.0	0.0
87473 Lung NAT (OD04451-02)	0.0	0.0
Normal Prostate Clontech A+ 6546-1	28.5	100.0
84140 Prostate Cancer (OD04410)	100.0	56.3
84141 Prostate NAT (OD04410)	23.5	27.2
87073 Prostate Cancer (OD04720-01)	15.7	28.3
87074 Prostate NAT (OD04720-02)	11.7	11.2
Normal Lung GENPAK 061010	0.0	4.8
83239 Lung Met to Muscle (ODO4286)	0.0	0.0
83240 Muscle NAT (ODO4286)	0.0	0.0
84136 Lung Malignant Cancer (OD03126)	8.2	5.3
84137 Lung NAT (OD03126)	0.0	0.0
84871 Lung Cancer (OD04404)	0.0	0.0
84872 Lung NAT (OD04404)	1.6	0.0
84875 Lung Cancer (OD04565)	0.0	0.0
84876 Lung NAT (OD04565)	0.0	0.0
85950 Lung Cancer (OD04237-01)	0.0	0.0
85970 Lung NAT (OD04237-02)	0.0	0.0
83255 Ocular Mel Met to Liver (ODO4310)	2.5	2.7
83256 Liver NAT (ODO4310)	0.0	0.0
84139 Melanoma Mets to Lung (OD04321)	3.6	0.0
84138 Lung NAT (OD04321)	0.0	0.0
Normal Kidney GENPAK 061008	0.0	0.0

83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	0.0
83787 Kidney NAT (OD04338)	0.0	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.0
83789 Kidney NAT (OD04339)	0.0	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	0.0
83791 Kidney NAT (OD04340)	0.0	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0
83793 Kidney NAT (OD04348)	0.0	4.6
87474 Kidney Cancer (OD04622-01)	0.0	0.0
87475 Kidney NAT (OD04622-03)	0.0	0.0
85973 Kidney Cancer (OD04450-01)	0.0	0.0
85974 Kidney NAT (OD04450-03)	0.0	0.0
Kidney Cancer Clontech 8120607	0.0	0.0
Kidney NAT Clontech 8120608	0.0	0.0
Kidney Cancer Clontech 8120613	0.0	0.0
Kidney NAT Clontech 8120614	2.2	0.0
Kidney Cancer Clontech 9010320	0.0	0.0
Kidney NAT Clontech 9010321	0.0	0.0
Normal Uterus GENPAK 061018	0.0	0.0
Uterus Cancer GENPAK 064011	0.0	0.0
Normal Thyroid Clontech A+ 6570-1	0.0	0.0
Thyroid Cancer GENPAK 064010	0.0	0.0
Thyroid Cancer INVITROGEN A302152	0.0	0.0
Thyroid NAT INVITROGEN A302153	0.0	0.0
Normal Breast GENPAK 061019	0.0	0.0
84877 Breast Cancer (OD04566)	0.0	0.0
85975 Breast Cancer (OD04590-01)	0.0	0.0
85976 Breast Cancer Mets (OD04590-03)	0.0	0.0
87070 Breast Cancer Metastasis (OD04655-05)	0.0	0.0
GENPAK Breast Cancer 064006	0.5	0.0
Breast Cancer Res. Gen. 1024	0.0	0.0
Breast Cancer Clontech 9100266	5.0	0.0
Breast NAT Clontech 9100265	1.8	2.9
Breast Cancer INVITROGEN A209073	3.1	0.0
Breast NAT INVITROGEN A2090734	0.0	0.0
Normal Liver GENPAK 061009	0.0	0.0
Liver Cancer GENPAK 064003	1.8	5.4
Liver Cancer Research Genetics RNA 1025	0.0	0.0
Liver Cancer Research Genetics RNA 1026	0.0	0.0
Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.0	0.0
Paired Liver Tissue Research Genetics RNA 6004-N	2.2	4.1
Paired Liver Cancer Tissue Research Genetics RNA 6005-T	4.0	0.0

Paired Liver Tissue Research Genetics RNA 6005-N	0.0	0.0
Normal Bladder GENPAK 061001	0.0	0.0
Bladder Cancer Research Genetics RNA 1023	0.0	0.0
Bladder Cancer INVITROGEN A302173	0.8	5.8
87071 Bladder Cancer (OD04718-01)	0.0	0.0
87072 Bladder Normal Adjacent (OD04718-03)	0.0	0.0
Normal Ovary Res. Gen.	0.0	0.0
Ovarian Cancer GENPAK 064008	0.0	0.0
87492 Ovary Cancer (OD04768-07)	0.0	0.0
87493 Ovary NAT (OD04768-08)	0.0	0.0
Normal Stomach GENPAK 061017	0.0	0.0
Gastric Cancer Clontech 9060358	0.0	0.0
NAT Stomach Clontech 9060359	0.0	0.0
Gastric Cancer Clontech 9060395	0.0	0.0
NAT Stomach Clontech 9060394	3.7	0.0
Gastric Cancer Clontech 9060397	0.0	0.0
NAT Stomach Clontech 9060396	0.0	0.0
Gastric Cancer GENPAK 064005	0.0	7.0

Panel 1.2 Summary Ag1523 Expression of the sggc_draft_ba656o22_20000731_da4 gene is highest in a melanoma cancer cell line (CT=26.5), with expression detected in a cluster of melanoma cell lines. Thus, the expression of this gene could be used to distinguish samples derived from melanoma cell lines from other samples. In addition, therapeutic modulation of the expression or function of this gene, through the use of small molecule drugs or antibodies might be of use in the treatment of melanoma. In addition, the sggc_draft_ba656o22_20000731_da4 gene is expressed in healthy prostate tissue but not in the prostate cancer cell line.

The sggc_draft_ba656o22_20000731_da4 gene is also expressed differentially in the cerebellum. A number of neurotransmitter effector systems are defective in the Alzheimer's disease brain including: defective G protein and protein kinase C function, drastically reduced level of receptors for the second messenger Ins(1,4,5) P3 and widespread impairment of G protein-stimulated adenylyl cyclase activity in Alzheimer's disease brain. Fowler C.J. et al., *Ann N Y Acad Sci.* 786:294-304 (1996); Cowburn R.F. et al., *J Neurochem.* 58:1409-19 (1992). Thus, cerebellum-preferential GPCR has utility as a drug target to counter the G-protein signaling deficit in Alzheimer's disease.

Panel 1.3D Summary Ag1898 Highest expression of the sggc_draft_ba656o22_20000731_da4 gene is detected in a melanoma cell line (CT=31) as is seen in Panel 1.2, with low but significant expression also seen in the cerebellum and testis. Thus, the expression of this gene could be

used to distinguish samples derived from this melanoma cell line from other samples. In addition, therapeutic modulation of the expression or function of this gene, through the use of small molecule drugs or antibodies, might be of use in the treatment of melanoma. Please see Panel 1.2 summary for potential relevance of expression in cerebellum to the treatment of CNS disorders.

Panel 2D Summary Ag1523 Two experiments with the same probe and primer set show expression of the *sggc_draft_ba656o22_20000731_da4* gene to be highest in a normal prostate in one run and a prostate cancer in the second run. In addition, the expression seen in both runs on panel 2D is specific to prostate derived samples. Thus, expression of the *sggc_draft_ba656o22_20000731_da4* gene could be used to distinguish samples derived from prostate tissue from other samples. Furthermore, since there is substantial over expression observed in a sample derived from prostate cancer when compared to a sample derived from its normal adjacent tissue, therapeutic modulation of the expression or function of the *sggc_draft_ba656o22_20000731_da4* gene product, through the use of small molecule drugs or antibodies, may be useful in the treatment of prostate cancer.

Panel 4D Summary Ag1898 Expression of the *sggc_draft_ba656o22_20000731_da4* gene is low/undetectable (Ct values >35) in all samples on this panel (data not shown).

GPCR4 (also referred to as AC0170103A_da1/CG54212-04 and CG54212-03)

Expression of gene AC0170103A_da1 and variant CG54212-03 was assessed using the primer-probe set Ag431, described in Table 18A. Results from RTQ-PCR runs are shown in Tables 18B, 18C, 18D, 18E, and 18F.

Table 18A Probe Name Ag431 (SEQ ID NO: 153, 154, 155)

Primers	Sequences	TM	Length	Start Position
Forward	5'-AGTCACTTCACCTGCAAGATCCT-3'		23	581
Probe	TET-5'-CCGCATGCCAGCTTCAGCACTG-3'-TAMRA		22	606
Reverse	5'-CTTCGCTGACCGACGTGTT-3'		19	629

Table 18B. Panel 1

Tissue Name	Relative Expression(%) tm550t	Tissue Name	Relative Expression(%) tm550t
Endothelial cells	0.0	Renal ca. 786-0	0.0
Endothelial cells (treated)	0.0	Renal ca. A498	1.5
Pancreas	0.0	Renal ca. RXF 393	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. ACHN	0.0
Adrenal gland	2.0	Renal ca. UO-31	0.0
Thyroid	0.0	Renal ca. TK-10	0.2

Salivary gland	0.0	Liver	0.1
Pituitary gland	0.4	Liver (fetal)	0.2
Brain (fetal)	27.5	Liver ca. (hepatoblast) HepG2	0.0
Brain (whole)	21.9	Lung	1.3
Brain (amygdala)	36.1	Lung (fetal)	0.6
Brain (cerebellum)	28.5	Lung ca. (small cell) LX-1	9.9
Brain (hippocampus)	36.1	Lung ca. (small cell) NCI- H69	18.4
Brain (substantia nigra)	17.1	Lung ca. (s.cell var.) SHP-77	0.0
Brain (thalamus)	36.1	Lung ca. (large cell) NCI- H460	5.3
Brain (hypothalamus)	13.5	Lung ca. (non-sm. cell) A549	8.4
Spinal cord	6.7	Lung ca. (non-s.cell) NCI- H23	0.4
CNS ca. (glio/astro) U87-MG	0.0	Lung ca (non-s.cell) HOP-62	0.0
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca. (non-s.cl) NCI-H522	5.8
CNS ca. (astro) SW1783	0.0	Lung ca. (squam.) SW 900	13.5
CNS ca.* (neuro; met) SK-N- AS	0.0	Lung ca. (squam.) NCI-H596	6.6
CNS ca. (astro) SF-539	0.0	Mammary gland	25.2
CNS ca. (astro) SNB-75	0.0	Breast ca.* (pl. effusion) MCF-7	0.9
CNS ca. (glio) SNB-19	2.6	Breast ca.* (pl.ef) MDA-MB- 231	0.0
CNS ca. (glio) U251	8.2	Breast ca.* (pl. effusion) T47D	4.3
CNS ca. (glio) SF-295	2.6	Breast ca. BT-549	0.3
Heart	4.0	Breast ca. MDA-N	0.2
Skeletal muscle	32.1	Ovary	0.4
Bone marrow	0.0	Ovarian ca. OVCAR-3	1.4
Thymus	2.5	Ovarian ca. OVCAR-4	0.0
Spleen	0.0	Ovarian ca. OVCAR-5	17.9
Lymph node	0.0	Ovarian ca. OVCAR-8	17.6
Colon (ascending)	16.0	Ovarian ca. IGROV-1	8.1
Stomach	0.6	Ovarian ca.* (ascites) SK- OV-3	0.9
Small intestine	0.5	Uterus	1.0
Colon ca. SW480	0.0	Placenta	0.5
Colon ca.* (SW480 met)SW620	0.0	Prostate	7.2
Colon ca. HT29	4.5	Prostate ca.* (bone met)PC-3	2.0
Colon ca. HCT-116	0.0	Testis	20.9
Colon ca. CaCo-2	0.0	Melanoma Hs688(A).T	0.0
Colon ca. HCT-15	10.5	Melanoma* (met) Hs688(B).T	1.6

Colon ca. HCC-2998	9.6	Melanoma UACC-62	1.9
Gastric ca.* (liver met) NCI-N87	8.5	Melanoma M14	0.0
Bladder	5.3	Melanoma LOX IMVI	0.0
Trachea	0.0	Melanoma* (met) SK-MEL-5	0.0
Kidney	1.0	Melanoma SK-MEL-28	100.0
Kidney (fetal)	3.2		

Table 18C. Panel 1.3D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	1.3Dtm3630t_ag431		1.3Dtm3630t_ag431
Liver adenocarcinoma	2.4	Kidney (fetal)	3.0
Pancreas	0.0	Renal ca. 786-0	0.0
Pancreatic ca. CAPAN 2	4.2	Renal ca. A498	0.0
Adrenal gland	0.0	Renal ca. RXF 393	0.0
Thyroid	0.0	Renal ca. ACHN	0.0
Salivary gland	0.0	Renal ca. UO-31	0.0
Pituitary gland	0.0	Renal ca. TK-10	0.0
Brain (fetal)	9.2	Liver	0.0
Brain (whole)	14.1	Liver (fetal)	0.0
Brain (amygdala)	24.8	Liver ca. (hepatoblast) HepG2	0.0
Brain (cerebellum)	16.7	Lung	0.0
Brain (hippocampus)	62.8	Lung (fetal)	11.7
Brain (substantia nigra)	2.5	Lung ca. (small cell) LX-1	9.6
Brain (thalamus)	5.4	Lung ca. (small cell) NCI-H69	0.0
Cerebral Cortex	36.3	Lung ca. (s.cell var.) SHP-77	3.2
Spinal cord	5.6	Lung ca. (large cell)NCI-H460	0.0
CNS ca. (glio/astro) U87-MG	0.0	Lung ca. (non-sm. cell) A549	0.0
CNS ca. (glio/astro) U-118-MG	0.0	Lung ca. (non-s.cell) NCI-H23	2.6
CNS ca. (astro) SW1783	0.0	Lung ca (non-s.cell) HOP-62	0.0
CNS ca.* (neuro; met) SK-N-AS	7.5	Lung ca. (non-s.cl) NCI-H522	2.9
CNS ca. (astro) SF-539	2.4	Lung ca. (squam.) SW 900	3.2
CNS ca. (astro) SNB-75	4.4	Lung ca. (squam.) NCI-H596	0.0
CNS ca. (glio) SNB-19	0.0	Mammary gland	4.4

CNS ca. (glio) U251	4.8	Breast ca.* (pl. effusion) MCF-7	0.0
CNS ca. (glio) SF-295	8.8	Breast ca.* (pl.ef) MDA-MB-231	2.6
Heart (fetal)	0.0	Breast ca.* (pl. effusion) T47D	0.0
Heart	2.9	Breast ca. BT-549	0.0
Fetal Skeletal	100.0	Breast ca. MDA-N	7.1
Skeletal muscle	6.3	Ovary	0.0
Bone marrow	0.0	Ovarian ca. OVCAR-3	0.0
Thymus	0.0	Ovarian ca. OVCAR-4	0.0
Spleen	3.3	Ovarian ca. OVCAR-5	0.0
Lymph node	3.0	Ovarian ca. OVCAR-8	0.0
Colorectal	19.2	Ovarian ca. IGROV-1	0.0
Stomach	0.0	Ovarian ca.* (ascites) SK-OV-3	0.0
Small intestine	0.0	Uterus	0.0
Colon ca. SW480	5.2	Placenta	0.0
Colon ca.* (SW480 met)SW620	0.0	Prostate	0.0
Colon ca. HT29	0.0	Prostate ca.* (bone met)PC-3	0.0
Colon ca. HCT-116	0.0	Testis	8.7
Colon ca. CaCo-2	0.0	Melanoma Hs688(A).T	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	Melanoma* (met) Hs688(B).T	0.0
Colon ca. HCC-2998	4.9	Melanoma UACC-62	2.7
Gastric ca.* (liver met) NCI-N87	9.7	Melanoma M14	0.0
Bladder	0.0	Melanoma LOX IMVI	0.0
Trachea	0.0	Melanoma* (met) SK-MEL-5	0.0
Kidney	4.7	Adipose	0.0

Table 18D. Panel 2D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	2Dtm3631t_ag431		2Dtm3631t_ag431
Normal Colon GENPAK 061003	2.0	Kidney NAT Clontech 8120608	0.0
83219 CC Well to Mod Diff (ODO3866)	1.4	Kidney Cancer Clontech 8120613	0.0
83220 CC NAT (ODO3866)	0.0	Kidney NAT Clontech 8120614	0.0
83221 CC Gr.2 rectosigmoid (ODO3868)	1.6	Kidney Cancer Clontech 9010320	0.0

83222 CC NAT (ODO3868)	2.0	Kidney NAT Clontech 9010321	20.7
83235 CC Mod Diff (ODO3920)	0.8	Normal Uterus GENPAK 061018	1.8
83236 CC NAT (ODO3920)	1.1	Uterus Cancer GENPAK 064011	4.9
83237 CC Gr.2 ascend colon (ODO3921)	1.9	Normal Thyroid Clontech A+ 6570-1	0.0
83238 CC NAT (ODO3921)	2.1	Thyroid Cancer GENPAK 064010	0.0
83241 CC from Partial Hepatectomy (ODO4309)	0.0	Thyroid Cancer INVITROGEN A302152	0.0
83242 Liver NAT (ODO4309)	1.2	Thyroid NAT INVITROGEN A302153	1.9
87472 Colon mets to lung (OD04451-01)	9.2	Normal Breast GENPAK 061019	0.9
87473 Lung NAT (OD04451-02)	0.7	84877 Breast Cancer (OD04566)	29.5
Normal Prostate Clontech A+ 6546-1	3.6	85975 Breast Cancer (OD04590-01)	2.8
84140 Prostate Cancer (OD04410)	3.1	85976 Breast Cancer Mets (OD04590-03)	0.0
84141 Prostate NAT (OD04410)	7.0	87070 Breast Cancer Metastasis (OD04655-05)	14.6
87073 Prostate Cancer (OD04720-01)	6.9	GENPAK Breast Cancer 064006	9.4
87074 Prostate NAT (OD04720-02)	7.9	Breast Cancer Res. Gen. 1024	100.0
Normal Lung GENPAK 061010	0.6	Breast Cancer Clontech 9100266	4.0
83239 Lung Met to Muscle (ODO4286)	0.0	Breast NAT Clontech 9100265	1.3
83240 Muscle NAT (ODO4286)	0.6	Breast Cancer INVITROGEN A209073	1.4
84136 Lung Malignant Cancer (OD03126)	2.0	Breast NAT INVITROGEN A2090734	2.7
84137 Lung NAT (OD03126)	4.2	Normal Liver GENPAK 061009	0.0
84871 Lung Cancer (OD04404)	0.0	Liver Cancer GENPAK 064003	0.7
84872 Lung NAT (OD04404)	0.9	Liver Cancer Research Genetics RNA 1025	0.0
84875 Lung Cancer (OD04565)	0.0	Liver Cancer Research Genetics RNA 1026	0.0
84876 Lung NAT (OD04565)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	2.2
85950 Lung Cancer (OD04237-01)	0.5	Paired Liver Tissue Research Genetics RNA 6004-N	2.4

85970 Lung NAT (OD04237-02)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0
83255 Ocular Mel Met to Liver (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
83256 Liver NAT (ODO4310)	0.0	Normal Bladder GENPAK 061001	3.6
84139 Melanoma Mets to Lung (OD04321)	0.0	Bladder Cancer Research Genetics RNA 1023	2.8
84138 Lung NAT (OD04321)	2.5	Bladder Cancer INVITROGEN A302173	2.8
Normal Kidney GENPAK 061008	2.5	87071 Bladder Cancer (OD04718-01)	0.8
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.9	87072 Bladder Normal Adjacent (OD04718-03)	3.2
83787 Kidney NAT (OD04338)	0.0	Normal Ovary Res. Gen.	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Ovarian Cancer GENPAK 064008	2.0
83789 Kidney NAT (OD04339)	3.1	87492 Ovary Cancer (OD04768-07)	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	87493 Ovary NAT (OD04768-08)	0.0
83791 Kidney NAT (OD04340)	0.0	Normal Stomach GENPAK 061017	4.8
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Gastric Cancer Clontech 9060358	0.0
83793 Kidney NAT (OD04348)	2.7	NAT Stomach Clontech 9060359	0.0
87474 Kidney Cancer (OD04622-01)	1.1	Gastric Cancer Clontech 9060395	2.9
87475 Kidney NAT (OD04622-03)	0.0	NAT Stomach Clontech 9060394	0.6
85973 Kidney Cancer (OD04450-01)	0.0	Gastric Cancer Clontech 9060397	0.6
85974 Kidney NAT (OD04450-03)	5.0	NAT Stomach Clontech 9060396	0.0
Kidney Cancer Clontech 8120607	0.0	Gastric Cancer GENPAK 064005	0.6

Table 18E. Panel 4D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	4Dtm3632t_ag431		4Dtm3632t_ag431
93768_Secondary Th1_anti-CD28/anti-CD3	0.0	93100_HUVEC (Endothelial)_IL-1b	0.0
93769_Secondary Th2_anti-CD28/anti-CD3	0.1	93779_HUVEC (Endothelial)_IFN gamma	0.1

93770_Secondary Tr1_anti-CD28/anti-CD3	0.0	93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	0.0
93573_Secondary Th1_resting day 4-6 in IL-2	0.0	93101_HUVEC (Endothelial)_TNF alpha + IL4	0.0
93572_Secondary Th2_resting day 4-6 in IL-2	0.0	93781_HUVEC (Endothelial)_IL-11	0.1
93571_Secondary Tr1_resting day 4-6 in IL-2	0.0	93583_Lung Microvascular Endothelial Cells none	0.0
93568_primary Th1_anti-CD28/anti-CD3	0.0	93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0
93569_primary Th2_anti-CD28/anti-CD3	0.2	92662_Microvascular Dermal endothelium_none	0.0
93570_primary Tr1_anti-CD28/anti-CD3	0.0	92663_Microvascular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0
93565_primary Th1_resting dy 4-6 in IL-2	0.0	93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.3
93566_primary Th2_resting dy 4-6 in IL-2	0.0	93347_Small Airway Epithelium none	0.0
93567_primary Tr1_resting dy 4-6 in IL-2	0.1	93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0
93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	0.0	92668_Coronary Artery SMC_resting	0.0
93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	0.0	92669_Coronary Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0
93251_CD8 Lymphocytes_anti-CD28/anti-CD3	0.0	93107_astrocytes_resting	0.0
93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	0.0	93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	0.0	92666_KU-812 (Basophil)_resting	0.0
93354_CD4 none	1.8	92667_KU-812 (Basophil)_PMA/ionoycin	0.0
93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	0.0	93579_CCD1106 (Keratinocytes)_none	0.0
93103_LAK cells_resting	0.0	93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	0.0
93788_LAK cells_IL-2	0.0	93791_Liver Cirrhosis	0.5
93787_LAK cells_IL-2+IL-12	0.0	93792_Lupus Kidney	0.0

93789_LAK cells_IL-2+IFN gamma	0.0	93577_NCI-H292	0.0
93790_LAK cells_IL-2+ IL-18	0.0	93358_NCI-H292_IL-4	0.4
93104_LAK cells_PMA/ionomycin and IL-18	0.1	93360_NCI-H292_IL-9	0.2
93578_NK Cells IL-2 resting	0.0	93359_NCI-H292_IL-13	0.0
93109_Mixed Lymphocyte Reaction_Two Way MLR	5.1	93357_NCI-H292_IFN gamma	100.0
93110_Mixed Lymphocyte Reaction_Two Way MLR	0.0	93777_HPAEC_-	0.0
93111_Mixed Lymphocyte Reaction_Two Way MLR	0.0	93778_HPAEC_IL-1 beta/TNA alpha	0.0
93112_Mononuclear Cells (PBMCs)_resting	0.0	93254_Normal Human Lung Fibroblast_none	0.0
93113_Mononuclear Cells (PBMCs)_PWM	0.0	93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0
93114_Mononuclear Cells (PBMCs)_PHA-L	0.0	93257_Normal Human Lung Fibroblast_IL-4	0.0
93249_Ramos (B cell)_none	0.0	93256_Normal Human Lung Fibroblast_IL-9	0.0
93250_Ramos (B cell)_ionomycin	0.0	93255_Normal Human Lung Fibroblast_IL-13	0.2
93349_B lymphocytes_PWM	0.1	93258_Normal Human Lung Fibroblast_IFN gamma	0.0
93350_B lymphocytes_CD40L and IL-4	0.4	93106_Dermal Fibroblasts CCD1070_resting	0.0
92665_EOL-1 (Eosinophil)_dbcAMP differentiated	0.0	93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	0.2
93248_EOL-1 (Eosinophil)_dbcAMP/PMAionomycin	0.0	93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	0.0
93356_Dendritic Cells_none	0.2	93772_dermal fibroblast_IFN gamma	0.0
93355_Dendritic Cells_LPS 100 ng/ml	0.0	93771_dermal fibroblast_IL-4	0.0
93775_Dendritic Cells_anti-CD40	0.0	93260_IBD Colitis 2	0.1
93774_Monocytes_resting	0.0	93261_IBD Crohns	0.0
93776_Monocytes_LPS 50 ng/ml	0.0	735010_Colon_normal	0.2
93581_Macrophages_resting	0.0	735019_Lung_none	0.2
93582_Macrophages_LPS 100 ng/ml	0.0	64028-1_Thymus_none	0.1
93098_HUVEC (Endothelial)_none	0.0	64030-1_Kidney_none	0.2

93099_HUVEC (Endothelial)_starved	0.0		
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Table 18F. Panel CNS_neurodegeneration_v1.0

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	tm6927t_ ag431_b1s1		tm6927t_ ag431_b1s1
AD 1 Hippo	4.4	Control (Path) 3 Temporal Ctx	10.1
AD 2 Hippo	7.4	Control (Path) 4 Temporal Ctx	43.0
AD 3 Hippo	8.2	AD 1 Occipital Ctx	29.3
AD 4 Hippo	7.6	AD 2 Occipital Ctx (Missing)	0.0
AD 5 Hippo	73.0	AD 3 Occipital Ctx	4.2
AD 6 Hippo	57.6	AD 4 Occipital Ctx	27.4
Control 2 Hippo	15.0	AD 5 Occipital Ctx	16.7
Control 4 Hippo	24.9	AD 6 Occipital Ctx	17.6
Control (Path) 3 Hippo	0.0	Control 1 Occipital Ctx	0.0
AD 1 Temporal Ctx	24.4	Control 2 Occipital Ctx	54.9
AD 2 Temporal Ctx	26.3	Control 3 Occipital Ctx	18.5
AD 3 Temporal Ctx	13.6	Control 4 Occipital Ctx	6.0
AD 4 Temporal Ctx	30.0	Control (Path) 1 Occipital Ctx	100.0
AD 5 Inf Temporal Ctx	54.9	Control (Path) 2 Occipital Ctx	8.8
AD 5 Sup Temporal Ctx	22.3	Control (Path) 3 Occipital Ctx	0.0
AD 6 Inf Temporal Ctx	80.3	Control (Path) 4 Occipital Ctx	32.2
AD 6 Sup Temporal Ctx	97.6	Control 1 Parietal Ctx	3.2
Control 1 Temporal Ctx	16.9	Control 2 Parietal Ctx	30.7
Control 2 Temporal Ctx	24.7	Control 3 Parietal Ctx	13.1
Control 3 Temporal Ctx	10.2	Control (Path) 1 Parietal Ctx	44.1
Control 3 Temporal Ctx	17.6	Control (Path) 2 Parietal Ctx	34.9
Control (Path) 1 Temporal Ctx	51.9	Control (Path) 3 Parietal Ctx	4.2
Control (Path) 2 Temporal Ctx	45.2	Control (Path) 4 Parietal Ctx	52.8

Panel 1 Summary Ag431 Expression of the AC0170103A_dal gene is highest in a melanoma cell line (CT=27.1). Significant expression is also detected in ovarian, lung, and colon cancer cell

lines. Moreover, therapeutic modulation of the expression or function of this gene, through the use of small molecule drugs or antibodies, might be of use in the treatment of melanoma and lung, colon or ovarian cancer.

Among tissues with metabolic function, the AC0170103A_da1 gene is expressed in the pituitary and adrenal glands, the hypothalamus, heart and skeletal muscle. Thus, the AC0170103A_da1 gene could be important in the pathogenesis and/or treatment of disease in any of those tissues.

The AC0170103A_da1 gene is also expressed at moderate levels in all of the tissues samples originating from the central nervous system including the fetal brain, cerebellum, amygdala, hippocampus, substantia nigra, thalamus, hypothalamus, and spinal cord. The protein encoded by the AC0170103A_da1 gene has homology to the GPCR family of receptors, to which several neurotransmitter receptors belong. Thus, this protein may represent a novel neurotransmitter receptor. Neurotransmitter receptors that are GPCRs include the dopamine receptor family, the serotonin receptor family, the GABAB receptor, and muscarinic acetylcholine receptors. The selected targeting of dopamine and serotonin receptors has proven to be effective in the treatment of psychiatric illnesses such as schizophrenia, bipolar disorder and depression. Furthermore, the cerebral cortex and hippocampus regions of the brain are known to play critical roles in Alzheimer's disease, seizure disorders, and in the normal process of memory formation. Therefore, therapeutic modulation of the AC0170103A_da1 gene or its protein product may be beneficial in the treatment of any of these diseases.

Panel 1.3D Summary Ag431 Expression of the AC0170103A_da1 gene occurs exclusively in fetal skeletal muscle in this panel (CT=34.5). Interestingly, this gene is not significantly expressed in adult skeletal tissue (CT=38.5), suggesting that AC0170103A_da1 gene expression could be used to distinguish between the two types of tissues. In addition, the relative overexpression of the AC0170103A_da1 gene in fetal skeletal muscle suggests that the protein product may enhance muscular growth or development in the fetus and thus may also act in a regenerative capacity in the adult. Therefore, therapeutic modulation of the AC0170103A_da1 gene could be useful in treatment of muscle related diseases. More specifically, treatment of weak or dystrophic muscle with the protein encoded by this gene could restore muscle mass or function.

Panel 2D Summary Ag431 Expression of the AC0170103A_da1 gene is highest in breast cancer (CT=32.3) and is not detected at significant levels in normal breast tissue. In addition, there is substantial expression in other samples derived from breast cancers. Thus, the expression of the

AC0170103A_dal gene could be used to distinguish breast cancer tissue from other samples. Moreover, therapeutic modulation of the protein encoded by the AC0170103A_dal gene, through the use of small molecule drugs or antibodies, might be of use in the treatment of breast cancer.

Panel 4D Summary Ag431 The AC0170103A_dal gene is expressed in IFN-gamma-stimulated mucoepidermoid (mucus-producing) NCI-H292 cells, but not in resting NCI-H292 cells, or in IL-4-, IL-9-, or IL-13-stimulated NCI-H292 cells. The gene is also expressed at low but significant levels at the three-day time point in a two-way mixed lymphocyte reaction with cells from normal human donors. Thus, inhibition of the function of the AC0170103A_dal gene product with antagonistic antibody or small molecule therapeutic may reduce or eliminate inflammation in colitis.

Panel CNS_neurodegeneration_v1.0 Summary Ag431 Expression of the AC0170103A_dal gene in this panel is low/undetectable (CT values >35) in all samples (data not shown).

GPCR5 (also referred to as 21629632.0.20)

Expression of gene 21629632.0.20 was assessed using the primer-probe set Ag1284, described in Tables 19A and 19B. Results from RTQ-PCR runs are shown in Tables 19C, 19D, 19E, 19F, 19G, 19H, and 19I.

Table 19A Probe Name Ag1284 (SEQ ID NO: 156, 157, 158)

Primers	Sequences	TM	Length	Start Position
Forward	5'-ACAACCCCATGTACTTCCTTCT-3'	58.9	22	197
Probe	TET-5'-AGCAACCTCTCCCTCATGGACATCTG-3'-TAMRA	69.7	26	220
Reverse	5'-GGGTTTCCAGGAAATTGTCTAG-3'	59	22	274

Table 19B. Probe Name 1539 (SEQ ID NO: 159, 160, 161)

Primers	Sequences	TM	Length	Start Position
Forward	5'-TTTATGGGACAATCTCCTTCA-3'	58.6	22	745
Probe	FAM-5'-TGTAATTCAAACCAAGGCCAAGGAT-3'-TAMRA	68.4	26	767
Reverse	5'-GAACAATGCGACAGTCTTATCC-3'	58.7	22	801

Table 19C. Panel 1.2

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
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	1.2tm2212f_ ag1539		1.2tm2212f_ ag1539
Endothelial cells	0.1	Renal ca. 786-0	0.7
Endothelial cells (treated)	3.5	Renal ca. A498	3.1
Pancreas	2.7	Renal ca. RXF 393	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. ACHN	3.4
Adrenal Gland (new lot*)	9.5	Renal ca. UO-31	2.2
Thyroid	1.3	Renal ca. TK-10	3.1
Salivary gland	26.1	Liver	2.8
Pituitary gland	2.1	Liver (fetal)	2.6
Brain (fetal)	4.9	Liver ca. (hepatoblast) HepG2	0.5
Brain (whole)	22.8	Lung	0.5
Brain (amygdala)	14.9	Lung (fetal)	0.8
Brain (cerebellum)	14.0	Lung ca. (small cell) LX-1	13.0
Brain (hippocampus)	81.2	Lung ca. (small cell) NCI-H69	2.0
Brain (thalamus)	31.9	Lung ca. (s.cell var.) SHP-77	0.1
Cerebral Cortex	100.0	Lung ca. (large cell) NCI-H460	2.8
Spinal cord	3.3	Lung ca. (non-sm. cell) A549	4.1
CNS ca. (glio/astro) U87-MG	1.4	Lung ca. (non-s.cell) NCI-H23	1.2
CNS ca. (glio/astro) U-118-MG	0.3	Lung ca (non-s.cell) HOP-62	8.4
CNS ca. (astro) SW1783	0.4	Lung ca. (non-s.cl) NCI-H522	23.3
CNS ca.* (neuro; met) SK-N-AS	1.7	Lung ca. (squam.) SW 900	13.8
CNS ca. (astro) SF-539	1.7	Lung ca. (squam.) NCI-H596	1.3
CNS ca. (astro) SNB-75	1.9	Mammary gland	6.6
CNS ca. (glio) SNB-19	5.0	Breast ca.* (pl. effusion) MCF-7	1.2
CNS ca. (glio) U251	3.1	Breast ca.* (pl.ef) MDA-MB-231	0.5
CNS ca. (glio) SF-295	25.9	Breast ca.* (pl. effusion) T47D	5.4
Heart	46.3	Breast ca. BT-549	37.4
Skeletal Muscle (new lot*)	52.1	Breast ca. MDA-N	1.3
Bone marrow	0.4	Ovary	7.1
Thymus	0.3	Ovarian ca. OVCAR-3	3.7
Spleen	1.2	Ovarian ca. OVCAR-4	1.8
Lymph node	0.6	Ovarian ca. OVCAR-5	27.7
Colorectal	0.2	Ovarian ca. OVCAR-8	6.6
Stomach	2.5	Ovarian ca. IGROV-1	5.7
Small intestine	7.1	Ovarian ca.* (ascites) SK-OV-3	3.4
Colon ca. SW480	0.3	Uterus	3.2
Colon ca.* (SW480 met)SW620	0.9	Placenta	0.4
Colon ca. HT29	1.5	Prostate	20.2
Colon ca. HCT-116	0.9	Prostate ca.* (bone met)PC-3	3.3

Colon ca. CaCo-2	2.3	Testis	1.3
83219 CC Well to Mod Diff (ODO3866)	0.6	Melanoma Hs688(A).T	0.6
Colon ca. HCC-2998	11.9	Melanoma* (met) Hs688(B).T	0.5
Gastric ca.* (liver met) NCI-N87	4.9	Melanoma UACC-62	3.9
Bladder	5.0	Melanoma M14	1.6
Trachea	0.2	Melanoma LOX IMVI	0.0
Kidney	30.4	Melanoma* (met) SK-MEL-5	0.0
Kidney (fetal)	11.5	Adipose	18.0

Table 19D. Panel 1.3D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	1.3Dtm2998f_ ag1539		1.3Dtm2998f_ ag1539
Liver adenocarcinoma	1.7	Kidney (fetal)	1.8
Pancreas	0.5	Renal ca. 786-0	1.6
Pancreatic ca. CAPAN 2	0.0	Renal ca. A498	2.2
Adrenal gland	2.7	Renal ca. RXF 393	0.5
Thyroid	4.4	Renal ca. ACHN	1.7
Salivary gland	2.0	Renal ca. UO-31	0.0
Pituitary gland	7.4	Renal ca. TK-10	1.2
Brain (fetal)	21.6	Liver	0.2
Brain (whole)	26.6	Liver (fetal)	1.6
Brain (amygdala)	30.8	Liver ca. (hepatoblast) HepG2	0.9
Brain (cerebellum)	7.6	Lung	1.7
Brain (hippocampus)	100.0	Lung (fetal)	3.5
Brain (substantia nigra)	5.0	Lung ca. (small cell) LX-1	4.1
Brain (thalamus)	15.8	Lung ca. (small cell) NCI-H69	1.2
Cerebral Cortex	76.8	Lung ca. (s.cell var.) SHP-77	0.0
Spinal cord	4.3	Lung ca. (large cell)NCI-H460	0.3
CNS ca. (glio/astro) U87-MG	0.0	Lung ca. (non-sm. cell) A549	2.1
CNS ca. (glio/astro) U-118-MG	0.3	Lung ca. (non-s.cell) NCI-H23	0.6
CNS ca. (astro) SW1783	0.7	Lung ca (non-s.cell) HOP-62	2.2

CNS ca.* (neuro; met) SK-N-AS	0.9	Lung ca. (non-s.cl) NCI-H522	4.0
CNS ca. (astro) SF-539	1.5	Lung ca. (squam.) SW 900	2.6
CNS ca. (astro) SNB-75	3.6	Lung ca. (squam.) NCI-H596	0.0
CNS ca. (glio) SNB-19	0.7	Mammary gland	1.8
CNS ca. (glio) U251	3.6	Breast ca.* (pl. effusion) MCF-7	0.0
CNS ca. (glio) SF-295	15.6	Breast ca.* (pl.ef) MDA-MB-231	0.9
Heart (fetal)	6.1	Breast ca.* (pl. effusion) T47D	1.6
Heart	2.4	Breast ca. BT-549	0.8
Fetal Skeletal	70.7	Breast ca. MDA-N	0.0
Skeletal muscle	0.5	Ovary	7.7
Bone marrow	0.0	Ovarian ca. OVCAR-3	1.0
Thymus	1.1	Ovarian ca. OVCAR-4	0.0
Spleen	0.4	Ovarian ca. OVCAR-5	4.8
Lymph node	1.0	Ovarian ca. OVCAR-8	1.8
Colorectal	8.5	Ovarian ca. IGROV-1	1.1
Stomach	2.9	Ovarian ca.* (ascites) SK-OV-3	0.6
Small intestine	4.5	Uterus	4.0
Colon ca. SW480	0.0	Placenta	0.3
Colon ca.* (SW480 met)SW620	0.9	Prostate	4.7
Colon ca. HT29	1.1	Prostate ca.* (bone met)PC-3	2.4
Colon ca. HCT-116	0.1	Testis	5.0
Colon ca. CaCo-2	0.9	Melanoma Hs688(A).T	1.3
<u>83219 CC Well to Mod</u> <u>Diff (ODO3866)</u>	1.2	Melanoma* (met) Hs688(B).T	1.8
Colon ca. HCC-2998	1.8	Melanoma UACC-62	0.7
Gastric ca.* (liver met) NCI-N87	3.3	Melanoma M14	0.3
Bladder	4.2	Melanoma LOX IMVI	0.0
Trachea	2.3	Melanoma* (met) SK-MEL-5	0.4
Kidney	3.3	Adipose	1.1

Table 19F. Panel 2D

Tissue Name	Relative Expression(%)	Relative Expression(%)
	2dx4tm4810t_ ag1284_a1	2dtm2829f_ ag1539
Normal Colon GENPAK 061003	0.0	37.9
83219 CC Well to Mod Diff (ODO3866)	0.0	2.7
83220 CC NAT (ODO3866)	0.0	2.7
83221 CC Gr.2 rectosigmoid (ODO3868)	0.0	7.2
83222 CC NAT (ODO3868)	0.0	3.0
83235 CC Mod Diff (ODO3920)	0.0	11.4
83236 CC NAT (ODO3920)	0.0	10.7
83237 CC Gr.2 ascend colon (ODO3921)	0.0	2.8
83238 CC NAT (ODO3921)	0.0	2.8
83241 CC from Partial Hepatectomy (ODO4309)	0.0	3.9
83242 Liver NAT (ODO4309)	0.0	0.3
87472 Colon mets to lung (OD04451-01)	0.0	7.5
87473 Lung NAT (OD04451-02)	0.0	4.3
Normal Prostate Clontech A+ 6546-1	0.0	0.0
84140 Prostate Cancer (OD04410)	0.0	10.8
84141 Prostate NAT (OD04410)	0.0	21.8
87073 Prostate Cancer (OD04720-01)	0.0	43.8
87074 Prostate NAT (OD04720-02)	0.0	19.8
Normal Lung GENPAK 061010	0.0	9.8
83239 Lung Met to Muscle (ODO4286)	0.0	0.0
83240 Muscle NAT (ODO4286)	0.0	5.4
84136 Lung Malignant Cancer (OD03126)	0.0	1.3
84137 Lung NAT (OD03126)	0.0	5.6
84871 Lung Cancer (OD04404)	0.0	0.8
84872 Lung NAT (OD04404)	0.0	5.0
84875 Lung Cancer (OD04565)	0.0	1.2
84876 Lung NAT (OD04565)	0.0	2.3
85950 Lung Cancer (OD04237-01)	0.0	6.0
85970 Lung NAT (OD04237-02)	0.0	4.9
83255 Ocular Mel Met to Liver (ODO4310)	0.0	1.4
83256 Liver NAT (ODO4310)	0.0	2.1
84139 Melanoma Mets to Lung (OD04321)	0.0	0.7
84138 Lung NAT (OD04321)	0.0	3.1
Normal Kidney GENPAK 061008	0.0	21.9
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	18.6
83787 Kidney NAT (OD04338)	0.0	10.5

83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	10.1
83789 Kidney NAT (OD04339)	0.0	16.8
83790 Kidney Ca, Clear cell type (OD04340)	0.0	6.2
83791 Kidney NAT (OD04340)	0.0	11.5
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0
83793 Kidney NAT (OD04348)	0.0	8.7
87474 Kidney Cancer (OD04622-01)	0.0	0.6
87475 Kidney NAT (OD04622-03)	0.0	0.8
85973 Kidney Cancer (OD04450-01)	0.0	5.0
85974 Kidney NAT (OD04450-03)	0.0	6.1
Kidney Cancer Clontech 8120607	0.0	3.5
Kidney NAT Clontech 8120608	0.0	1.1
Kidney Cancer Clontech 8120613	0.0	2.8
Kidney NAT Clontech 8120614	0.0	5.4
Kidney Cancer Clontech 9010320	0.0	1.9
Kidney NAT Clontech 9010321	0.0	8.6
Normal Uterus GENPAK 061018	0.0	1.4
Uterus Cancer GENPAK 064011	0.0	17.0
Normal Thyroid Clontech A+ 6570-1	0.0	6.8
Thyroid Cancer GENPAK 064010	0.0	4.0
Thyroid Cancer INVITROGEN A302152	0.0	7.9
Thyroid NAT INVITROGEN A302153	0.0	9.0
Normal Breast GENPAK 061019	0.0	16.0
84877 Breast Cancer (OD04566)	0.0	40.1
85975 Breast Cancer (OD04590-01)	0.0	17.8
85976 Breast Cancer Mets (OD04590-03)	0.0	12.3
87070 Breast Cancer Metastasis (OD04655-05)	0.0	23.2
GENPAK Breast Cancer 064006	0.0	15.8
Breast Cancer Res. Gen. 1024	0.0	100.0
Breast Cancer Clontech 9100266	0.0	7.1
Breast NAT Clontech 9100265	0.0	8.2
Breast Cancer INVITROGEN A209073	100.0	19.2
Breast NAT INVITROGEN A2090734	0.0	11.9
Normal Liver GENPAK 061009	0.0	3.8
Liver Cancer GENPAK 064003	0.0	1.2
Liver Cancer Research Genetics RNA 1025	0.0	3.7
Liver Cancer Research Genetics RNA 1026	0.0	1.4
Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.0	3.0
Paired Liver Tissue Research Genetics RNA 6004-N	0.0	0.6
Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0	0.5
Paired Liver Tissue Research Genetics RNA 6005-N	0.0	0.3
Normal Bladder GENPAK 061001	0.0	7.7

Bladder Cancer Research Genetics RNA 1023	0.0	2.3
Bladder Cancer INVITROGEN A302173	0.0	3.0
87071 Bladder Cancer (OD04718-01)	0.0	1.3
87072 Bladder Normal Adjacent (OD04718-03)	0.0	19.1
Normal Ovary Res. Gen.	0.0	3.6
Ovarian Cancer GENPAK 064008	0.0	10.0
87492 Ovary Cancer (OD04768-07)	0.0	3.7
87493 Ovary NAT (OD04768-08)	0.0	1.9
Normal Stomach GENPAK 061017	0.0	15.4
Gastric Cancer Clontech 9060358	0.0	2.9
NAT Stomach Clontech 9060359	0.0	2.1
Gastric Cancer Clontech 9060395	0.0	8.2
NAT Stomach Clontech 9060394	0.0	4.2
Gastric Cancer Clontech 9060397	0.0	5.1
NAT Stomach Clontech 9060396	0.0	1.4
Gastric Cancer GENPAK 064005	0.0	6.8

Table 19G. Panel 4.1D

Tissue Name	Relative Expression(%) 4.1x4tm6516f ag1539_a1	Tissue Name	Relative Expression(%) 4.1x4tm6516f ag1539_a1
93768_Secondary Th1_anti-CD28/anti-CD3	0.0	93100_HUVEC (Endothelial)_IL-1b	0.0
93769_Secondary Th2_anti-CD28/anti-CD3	0.0	93779_HUVEC (Endothelial)_IFN gamma	0.0
93770_Secondary Tr1_anti-CD28/anti-CD3	0.0	93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	0.0
93573_Secondary Th1_resting day 4-6 in IL-2	0.5	93101_HUVEC (Endothelial)_TNF alpha + IL4	0.0
93572_Secondary Th2_resting day 4-6 in IL-2	0.9	93781_HUVEC (Endothelial)_IL-11	0.0
93571_Secondary Tr1_resting day 4-6 in IL-2	0.6	93583_Lung Microvascular Endothelial Cells_none	0.7
93568_primary Th1_anti-CD28/anti-CD3	0.2	93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.2
93569_primary Th2_anti-CD28/anti-CD3	0.7	92662_Microvascular Dermal endothelium_none	0.3
93570_primary Tr1_anti-CD28/anti-CD3	0.0	92663_Microvascular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0
93565_primary Th1_resting dy 4-6 in IL-2	0.0	93773_Bronchial epithelium_TNFa (4 ng/ml) and	3.6

		IL1b (1 ng/ml) **	
93566_primary Th2_resting dy 4-6 in IL-2	0.0	93347_Small Airway Epithelium_none	0.7
93567_primary Tr1_resting dy 4-6 in IL-2	1.1	93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.9
93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	1.4	92668_Coronary Artery SMC_resting	0.4
93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	1.6	92669_Coronary Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.7
93251_CD8 Lymphocytes_anti-CD28/anti-CD3	0.0	93107_astrocytes_resting	6.1
93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	0.6	93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	2.8
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	1.1	92666_KU-812 (Basophil)_resting	0.0
93354_CD4_none	2.9	92667_KU-812 (Basophil)_PMA/ionoycin	0.0
93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	1.3	93579_CCD1106 (Keratinocytes)_none	0.7
93103_LAK cells_resting	1.5	93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	0.7
93788_LAK cells_IL-2	1.6	93791_Liver Cirrhosis	0.8
93787_LAK cells_IL-2+IL-12	0.4	93577_NCI-H292	5.3
93789_LAK cells_IL-2+IFN gamma	2.1	93358_NCI-H292_IL-4	2.7
93790_LAK cells_IL-2+ IL-18	2.0	93360_NCI-H292_IL-9	5.6
93104_LAK cells_PMA/ionomycin and IL-18	0.2	93359_NCI-H292_IL-13	0.0
93578_NK Cells_IL-2_resting	0.4	93357_NCI-H292_IFN gamma	0.8
93109_Mixed Lymphocyte Reaction_Two Way MLR	2.6	93777_HPAEC_-	0.0
93110_Mixed Lymphocyte Reaction_Two Way MLR	2.2	93778_HPAEC_IL-1 beta/TNA alpha	0.0
93111_Mixed Lymphocyte Reaction_Two Way MLR	0.4	93254_Normal Human Lung Fibroblast_none	8.5
93112_Mononuclear Cells (PBMCs)_resting	0.5	93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.3
93113_Mononuclear Cells (PBMCs)_PWM	0.0	93257_Normal Human Lung Fibroblast_IL-4	0.8
93114_Mononuclear Cells (PBMCs)_PHA-L	0.0	93256_Normal Human Lung Fibroblast_IL-9	3.1

93249 Ramos (B cell) none	0.0	93255 Normal Human Lung Fibroblast IL-13	0.5
93250 Ramos (B cell) ionomycin	0.0	93258 Normal Human Lung Fibroblast IFN gamma	1.6
93349 B lymphocytes PWM	0.0	93106 Dermal Fibroblasts CCD1070 resting	0.0
93350 B lymphocytes_CD40L and IL-4	1.1	93361 Dermal Fibroblasts CCD1070 TNF alpha 4 ng/ml	1.0
92665 EOL-1 (Eosinophil) dbcAMP differentiated	0.8	93105 Dermal Fibroblasts CCD1070 IL-1 beta 1 ng/ml	1.3
93248 EOL-1 (Eosinophil) dbcAMP/PMAionomycin	0.0	93772 dermal fibroblast IFN gamma	3.3
93356 Dendritic Cells none	0.4	93771 dermal fibroblast IL-4	2.7
93355 Dendritic Cells LPS 100 ng/ml	0.0	93892 Dermal fibroblasts none	4.1
93775 Dendritic Cells anti-CD40	0.0	99202 Neutrophils TNFa+LPS	0.4
93774 Monocytes resting	1.3	99203 Neutrophils none	1.2
93776 Monocytes LPS 50 ng/ml	0.3	735010 Colon normal	4.4
93581 Macrophages resting	0.3	735019 Lung none	5.6
93582 Macrophages LPS 100 ng/ml	0.0	64028-1 Thymus none	25.8
93098 HUVEC (Endothelial) none	0.0	64030-1 Kidney none	100.0
93099 HUVEC (Endothelial) starved	0.0		

Table 19H. Panel CNSD.01

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	cns_1x4tm654 8f_ag1539_a2		cns_1x4tm654 8f_ag1539_a2
102633 BA4 Control	29.0	102605 BA17 PSP	35.0
102641 BA4 Control2	39.6	102612 BA17 PSP2	17.3
102625 BA4 Alzheimer's2	19.1	102637 Sub Nigra Control	29.8
102649 BA4 Parkinson's	69.4	102645 Sub Nigra Control2	10.3
102656 BA4 Parkinson's2	62.4	102629 Sub Nigra Alzheimer's2	10.7
102664 BA4 Huntington's	21.0	102660 Sub Nigra Parkinson's2	26.1
102671 BA4 Huntington's2	8.5	102667 Sub Nigra Huntington's	65.0
102603 BA4 PSP	19.8	102674 Sub Nigra Huntington's2	11.5
102610 BA4 PSP2	18.2	102614 Sub Nigra PSP2	0.0
102588 BA4 Depression	27.0	102592 Sub Nigra Depression	7.0

102596_BA4 Depression2	17.5	102599_Sub Nigra Depression2	5.0
102634_BA7 Control	53.0	102636_Glob Palladus Control	19.8
102642_BA7 Control2	58.2	102644_Glob Palladus Control2	12.3
102626_BA7 Alzheimer's2	18.5	102620_Glob Palladus Alzheimer's	8.8
102650_BA7 Parkinson's	35.1	102628_Glob Palladus Alzheimer's2	49.1
102657_BA7 Parkinson's2	53.0	102652_Glob Palladus Parkinson's	89.9
102665_BA7 Huntington's	72.5	102659_Glob Palladus Parkinson's2	9.6
102672_BA7 Huntington's2	34.3	102606_Glob Palladus PSP	8.2
102604_BA7 PSP	70.3	102613_Glob Palladus PSP2	4.1
102611_BA7 PSP2	30.1	102591_Glob Palladus Depression	17.4
102589_BA7 Depression	14.3	102638_Temp Pole Control	7.1
102632_BA9 Control	34.9	102646_Temp Pole Control2	75.9
102640_BA9 Control2	73.9	102622_Temp Pole Alzheimer's	9.4
102617_BA9 Alzheimer's	15.5	102630_Temp Pole Alzheimer's2	17.1
102624_BA9 Alzheimer's2	19.8	102653_Temp Pole Parkinson's	38.3
102648_BA9 Parkinson's	58.0	102661_Temp Pole Parkinson's2	38.8
102655_BA9 Parkinson's2	66.2	102668_Temp Pole Huntington's	45.6
102663_BA9 Huntington's	52.5	102607_Temp Pole PSP	14.7
102670_BA9 Huntington's2	34.9	102615_Temp Pole PSP2	21.3
102602_BA9 PSP	21.1	102600_Temp Pole Depression2	9.0
102609_BA9 PSP2	6.9	102639_Cing Gyr Control	39.0
102587_BA9 Depression	20.9	102647_Cing Gyr Control2	48.6
102595_BA9 Depression2	9.6	102623_Cing Gyr Alzheimer's	12.4
102635_BA17 Control	74.2	102631_Cing Gyr Alzheimer's2	11.1
102643_BA17 Control2	100.0	102654_Cing Gyr Parkinson's	18.0
102627_BA17 Alzheimer's2	23.3	102662_Cing Gyr Parkinson's2	32.8
102651_BA17 Parkinson's	82.8	102669_Cing Gyr Huntington's	81.6
102658_BA17 Parkinson's2	91.3	102676_Cing Gyr Huntington's2	23.9
102666_BA17 Huntington's	59.8	102608_Cing Gyr PSP	19.6
102673_BA17 Huntington's2	36.6	102616_Cing Gyr PSP2	7.1
102590_BA17 Depression	31.9	102594_Cing Gyr Depression	19.1
102597_BA17 Depression2	46.3	102601_Cing Gyr Depression2	14.9

Table 19I. Panel CNS_neurodegeneration_v1.0

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	tm6956f_ag1539_a2s2		tm6956f_ag1539_a2s2
AD 1 Hippo	22.2	Control (Path) 3 Temporal Ctx	11.5
AD 2 Hippo	40.9	Control (Path) 4 Temporal Ctx	58.4
AD 3 Hippo	14.8	AD 1 Occipital Ctx	29.5
AD 4 Hippo	19.2	AD 2 Occipital Ctx (Missing)	0.0
AD 5 hippo	90.4	AD 3 Occipital Ctx	11.4
AD 6 Hippo	66.6	AD 4 Occipital Ctx	35.1
Control 2 Hippo	41.4	AD 5 Occipital Ctx	20.7
Control 4 Hippo	18.6	AD 6 Occipital Ctx	46.0
Control (Path) 3 Hippo	6.1	Control 1 Occipital Ctx	7.6
AD 1 Temporal Ctx	27.2	Control 2 Occipital Ctx	52.7
AD 2 Temporal Ctx	44.2	Control 3 Occipital Ctx	37.4
AD 3 Temporal Ctx	19.2	Control 4 Occipital Ctx	14.4
AD 4 Temporal Ctx	39.8	Control (Path) 1 Occipital Ctx	100.0
AD 5 Inf Temporal Ctx	78.4	Control (Path) 2 Occipital Ctx	28.4
AD 5 Sup Temporal Ctx	49.6	Control (Path) 3 Occipital Ctx	4.9
AD 6 Inf Temporal Ctx	69.7	Control (Path) 4 Occipital Ctx	39.2
AD 6 Sup Temporal Ctx	70.8	Control 1 Parietal Ctx	15.9
Control 1 Temporal Ctx	11.6	Control 2 Parietal Ctx	50.3
Control 2 Temporal Ctx	40.0	Control 3 Parietal Ctx	25.2
Control 3 Temporal Ctx	29.0	Control (Path) 1 Parietal Ctx	83.5
Control 4 Temporal Ctx	16.8	Control (Path) 2 Parietal Ctx	42.6
Control (Path) 1 Temporal Ctx	85.6	Control (Path) 3 Parietal Ctx	7.7
Control (Path) 2 Temporal Ctx	57.9	Control (Path) 4 Parietal Ctx	71.3

Panel 1.2 Summary Ag1539 The 21629632.0.2 gene shows rather ubiquitous expression across the samples on this panel, with highest expression in cerebral cortex (CT=25) and hippocampus. See Panel 1.3D summary for explanation.

Panel 1.3D Summary Ag1539 The expression of the 21629632.0.2 gene is highest in the samples of brain tissue and fetal muscle. The latter profile is of particular interest in that it differs significantly from that of the adult skeletal muscle. This difference implies that this protein may function to enhance muscular growth or development in the fetus and thus may also act in a regenerative capacity in the adult. Thus, therapeutic modulation of this gene could be useful in treatment of muscular related disease. For instance treatment of weak or dystrophic muscle with the protein encoded by this gene could restore muscle mass or function.

The 21629632.0.2 transcript also shows highly preferential expression in brain, especially in the hippocampus and cerebral cortex where the expression is fairly high (CT = 29.5). The protein encoded by the 21629632.0.2 gene appears to be a GPCR, making it an excellent small molecule target. Both the hippocampus and cerebral cortex are affected by neurodegeneration in Alzheimer's disease; thus

this molecule is an excellent candidate for a drug target for the treatment/prevention of Alzheimer's disease, and may also be useful for memory enhancement/processing in healthy subjects.

Panel 2D Summary Ag1539/Ag1284 Two experiments with two different probe and primer sets show significant expression of the 21629632.0.2 gene in breast cancer and prostate cancer. Thus, the expression of this gene could be used to distinguish a sample of breast cancer form other tissues. Moreover, therapeutic modulation of the expression or function of the 21629632.0.2 gene product, through the use of small molecule drugs or antibodies, may be useful in the treatment of breast cancer.

Panel 4.1D Summary Ag1539 The 21629632.0.2 gene is expressed at high levels in the kidney and at somewhat lower levels in the thymus. The 21629632.0.2 gene, the protein encoded for by the gene, or antibodies designed with the protein could be used to identify kidney and thymus tissue.

Panel CNSD.01 Summary Ag1539 An examination of the 21629632.0.2 gene expression in 8 brain regions across 12 individuals confirms that this protein is expressed in the brain of most, if not all, individuals including those suffering from neurologic/psychiatric disease. Utility as a drug target would benefit from likely expression in most disease states.

Panel CNS_neurodegeneration_v1.0 Ag1539 The 21629632.0.2 gene encodes a protein with homology to the GPCR family of receptors. In this panel, the gene is expressed in the brain, although no association of gene expression level with Alzheimer's disease was detected. Several neurotransmitter receptors are GPCRs, including the dopamine receptor family, the serotonin receptor family, the GABAB receptor, muscarinic acetylcholine receptors, and others; thus this GPCR may represent a novel neurotransmitter receptor. Targeting various neurotransmitter receptors (dopamine, serotonin) has proven to be an effective therapy in psychiatric illnesses such as schizophrenia, bipolar disorder and depression. Furthermore the cerebral cortex and hippocampus are regions of the brain that are known to play critical roles in Alzheimer's disease, seizure disorders, and in the normal process of memory formation. Therefore, therapeutic modulation of the 21629632.0.2 gene or its protein product may be beneficial in the treatment of one or more of these diseases, as may stimulation of the receptor coded for by the gene.

GPCR6 (also refered to as CG50177-01)

Expression of gene CG50177-01 was assessed using the primer-probe sets Ag2385, Ag1609, Ag1223, and Ag2320, described in Tables 20A, 20B, and 20C. Results from RTQ-PCR runs are shown in Tables 20D, 20E, 20F, and 20G.

Table 20A Probe Name Ag2385 (SEQ ID NO: 162, 163, 164)

Primers	Sequences	TM	Length	Start Position
Forward	5'-CACAACTGTGGTCATCTCTTCA-3'	58.8	22	580
Probe	TET-5'-CCCTCATGTCCACATCCTCCTTACCA-3'-TAMRA	70	26	631

Reverse	5'-GGTGGGAAGAGCAGATAGAAAT-3'	58.7	22	657
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Table 20B Probe Name Ag1609/1223 (identical sequences) (SEQ ID NO: 165, 166, 167)

Primers	Sequences	TM	Length	Start Position
Forward	5'-TAACACATCCAACCTGCCTTCTT-3'	58.8	22	37
Probe	FAM-5'-AGGCCTGGAACACCTGCACATCT-3'-TAMRA	68.5	23	74
Reverse	5'-CTAAGCAGAAAGGGATGGAGAT-3'	58.9	22	99

5 **Table 20C** Probe Name Ag2320 (SEQ ID NO: 168, 169, 170)

Primers	Sequences	TM	Length	Start Position
Forward	5'-TCTTCAGGCAGTTCTACTGCTT-3'	58.4	22	700
Probe	FAM-5'-CTCTCAGGAGGCCCGCTACAAGG-3'-TAMRA	69.4	23	724
Reverse	5'-TGGCACCTATATGAGAGACACA-3'	58.3	22	758

Table 20D. Panel 1.3D

Tissue Name	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)
	1.3dx4tm5464 t ag2385 b2	1.3dx4tm5437 f ag2320 a1	1.3dtm2773 f ag1223	1.3dx4tm5415 t ag1609 a2
Liver adenocarcinoma	0.0	0.0	0.0	0.0
Pancreas	0.0	0.0	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0	0.0	0.0
Adrenal gland	0.0	0.0	1.5	0.0
Thyroid	0.0	0.0	0.0	0.0
Salivary gland	0.0	0.0	1.0	0.0
Pituitary gland	0.0	0.0	0.0	0.0
Brain (fetal)	0.0	0.0	0.0	0.0
Brain (whole)	0.0	46.5	0.0	0.0
Brain (amygdala)	0.0	0.0	4.6	25.6
Brain (cerebellum)	0.0	0.0	0.0	0.0
Brain (hippocampus)	0.0	0.0	9.4	0.0
Brain (substantia nigra)	0.0	17.0	0.0	17.7
Brain (thalamus)	0.0	10.7	0.0	0.0
Cerebral Cortex	0.0	0.0	0.0	0.0
Spinal cord	0.0	13.0	3.3	23.3
CNS ca. (glio/astro) U87-MG	0.0	0.0	0.0	0.0
CNS ca. (glio/astro) U-118-MG	0.0	0.0	0.5	0.0
CNS ca. (astro) SW1783	0.0	0.0	9.9	0.0

CNS ca.* (neuro; met) SK-N-AS	0.0	0.0	0.0	0.0
CNS ca. (astro) SF-539	0.0	15.7	2.1	0.0
CNS ca. (astro) SNB-75	0.0	0.0	0.0	0.0
CNS ca. (glio) SNB-19	0.0	0.0	1.9	0.0
CNS ca. (glio) U251	3.8	0.0	1.9	0.0
CNS ca. (glio) SF-295	0.0	0.0	0.0	0.0
Heart (fetal)	0.0	0.0	0.0	0.0
Heart	0.0	0.0	0.0	0.0
Fetal Skeletal	0.0	0.0	11.1	0.0
Skeletal muscle	0.0	0.0	0.0	0.0
Bone marrow	0.0	32.5	24.7	29.9
Thymus	0.0	0.0	0.0	0.0
Spleen	0.0	0.0	6.2	22.3
Lymph node	0.0	13.7	1.9	15.2
Colorectal	4.2	0.0	2.4	0.0
Stomach	0.0	0.0	0.0	0.0
Small intestine	0.0	10.5	0.0	0.0
Colon ca. SW480	0.0	0.0	0.0	0.0
Colon ca.* (SW480 met)SW620	0.0	0.0	0.0	0.0
Colon ca. HT29	0.0	0.0	0.0	0.0
Colon ca. HCT-116	0.0	0.0	0.0	0.0
Colon ca. CaCo-2	0.0	0.0	0.0	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	0.0	6.2	0.0
Colon ca. HCC-2998	0.0	7.0	2.0	0.0
Gastric ca.* (liver met) NCI-N87	0.0	100.0	100.0	100.0
Bladder	0.0	0.0	0.0	0.8
Trachea	0.0	10.5	1.3	0.0
Kidney	0.0	0.0	0.0	0.0
Kidney (fetal)	0.0	0.0	0.0	0.0
Renal ca. 786-0	0.0	0.0	0.0	0.0
Renal ca. A498	0.0	0.0	0.0	0.0
Renal ca. RXF 393	0.0	0.0	0.0	0.0
Renal ca. ACHN	0.0	5.7	0.0	0.0
Renal ca. UO-31	0.0	0.0	0.0	0.0
Renal ca. TK-10	3.3	0.0	0.0	0.0
Liver	0.0	0.0	0.0	0.0
Liver (fetal)	0.0	23.5	0.0	7.1
Liver ca. (hepatoblast) HepG2	0.0	0.0	0.0	0.0
Lung	0.0	11.6	0.0	1.0
Lung (fetal)	0.0	18.3	8.1	8.9

Lung ca. (small cell) LX-1	0.0	0.0	0.0	0.0
Lung ca. (small cell) NCI-H69	25.3	0.0	0.0	0.0
Lung ca. (s.cell var.) SHP-77	100.0	0.0	0.0	0.0
Lung ca. (large cell) NCI-H460	5.8	0.0	0.0	0.0
Lung ca. (non-sm. cell) A549	0.0	0.0	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	2.6	0.0	0.0	0.0
Lung ca (non-s.cell) HOP-62	0.0	0.0	0.0	0.0
Lung ca. (non-s.cl) NCI-H522	0.0	4.6	0.0	0.0
Lung ca. (squam.) SW 900	0.0	0.0	1.9	0.0
Lung ca. (squam.) NCI-H596	24.8	0.0	0.0	0.0
Mammary gland	0.0	0.0	0.0	0.0
Breast ca.* (pl. effusion) MCF-7	0.0	0.0	0.0	0.0
Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0	0.0	0.0
Breast ca.* (pl. effusion) T47D	0.0	0.0	0.0	0.0
Breast ca. BT-549	0.0	0.0	0.0	0.0
Breast ca. MDA-N	0.0	0.0	0.0	0.0
Ovary	0.0	0.0	4.2	0.0
Ovarian ca. OVCAR-3	0.0	0.0	1.7	0.0
Ovarian ca. OVCAR-4	0.0	0.0	0.0	0.0
Ovarian ca. OVCAR-5	0.0	0.0	1.5	0.0
Ovarian ca. OVCAR-8	0.0	0.0	0.0	0.0
Ovarian ca. IGROV-1	0.0	0.0	0.0	0.0
Ovarian ca.* (ascites) SK-OV-3	0.0	0.0	0.0	0.0
Uterus	0.0	0.0	0.0	0.0
Placenta	0.0	0.0	0.0	0.0
Prostate	5.1	0.0	1.8	0.0
Prostate ca.* (bone met) PC-3	0.0	0.0	0.0	0.0
Testis	0.0	0.0	0.0	0.0
Melanoma Hs688(A).T	0.0	0.0	0.0	0.0
Melanoma* (met) Hs688(B).T	0.0	0.0	0.0	0.0
Melanoma UACC-62	0.0	0.0	0.0	0.0
Melanoma M14	0.0	0.0	0.0	0.0
Melanoma LOX IMVI	0.0	0.0	0.0	0.0
Melanoma* (met) SK-MEL-5	0.0	0.0	0.0	0.0
Adipose	0.0	6.1	3.9	0.0

Table 20E. Panel 2D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
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	2dx4tm4719f_ ag1223_a1		2dx4tm4719f_ ag1223_a1
Normal Colon GENPAK 061003	21.2	Kidney NAT Clontech 8120608	0.0
83219 CC Well to Mod Diff (ODO3866)	8.9	Kidney Cancer Clontech 8120613	0.0
83220 CC NAT (ODO3866)	10.0	Kidney NAT Clontech 8120614	0.0
83221 CC Gr.2 rectosigmoid (ODO3868)	0.0	Kidney Cancer Clontech 9010320	9.0
83222 CC NAT (ODO3868)	0.0	Kidney NAT Clontech 9010321	5.3
83235 CC Mod Diff (ODO3920)	5.7	Normal Uterus GENPAK 061018	0.0
83236 CC NAT (ODO3920)	2.8	Uterus Cancer GENPAK 064011	7.4
83237 CC Gr.2 ascend colon (ODO3921)	2.6	Normal Thyroid Clontech A+ 6570-1	0.0
83238 CC NAT (ODO3921)	12.8	Thyroid Cancer GENPAK 064010	5.4
83241 CC from Partial Hepatectomy (ODO4309)	17.4	Thyroid Cancer INVITROGEN A302152	0.0
83242 Liver NAT (ODO4309)	9.3	Thyroid NAT INVITROGEN A302153	7.5
87472 Colon mets to lung (OD04451-01)	4.5	Normal Breast GENPAK 061019	6.2
87473 Lung NAT (OD04451-02)	2.7	84877 Breast Cancer (OD04566)	25.2
Normal Prostate Clontech A+ 6546-1	11.0	85975 Breast Cancer (OD04590-01)	20.0
84140 Prostate Cancer (OD04410)	3.1	85976 Breast Cancer Mets (OD04590-03)	27.3
84141 Prostate NAT (OD04410)	7.4	87070 Breast Cancer Metastasis (OD04655-05)	19.6
87073 Prostate Cancer (OD04720-01)	2.3	GENPAK Breast Cancer 064006	12.1
87074 Prostate NAT (OD04720-02)	23.2	Breast Cancer Res. Gen. 1024	0.0
Normal Lung GENPAK 061010	100.0	Breast Cancer Clontech 9100266	8.0
83239 Lung Met to Muscle (ODO4286)	6.9	Breast NAT Clontech 9100265	2.6
83240 Muscle NAT (ODO4286)	2.5	Breast Cancer INVITROGEN A209073	2.5
84136 Lung Malignant Cancer (OD03126)	18.2	Breast NAT INVITROGEN A2090734	0.0
84137 Lung NAT (OD03126)	22.7	Normal Liver GENPAK 061009	7.7
84871 Lung Cancer (OD04404)	10.6	Liver Cancer GENPAK 064003	6.0
84872 Lung NAT (OD04404)	7.8	Liver Cancer Research Genetics RNA 1025	10.6

84875 Lung Cancer (OD04565)	2.8	Liver Cancer Research Genetics RNA 1026	13.1
84876 Lung NAT (OD04565)	0.7	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	4.0
85950 Lung Cancer (OD04237-01)	9.5	Paired Liver Tissue Research Genetics RNA 6004-N	17.8
85970 Lung NAT (OD04237-02)	22.4	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	7.9
83255 Ocular Mel Met to Liver (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
83256 Liver NAT (ODO4310)	4.6	Normal Bladder GENPAK 061001	34.1
84139 Melanoma Mets to Lung (OD04321)	0.0	Bladder Cancer Research Genetics RNA 1023	11.5
84138 Lung NAT (OD04321)	13.5	Bladder Cancer INVITROGEN A302173	7.1
Normal Kidney GENPAK 061008	2.8	87071 Bladder Cancer (OD04718-01)	0.6
83786 Kidney Ca, Nuclear grade 2 (OD04338)	22.6	87072 Bladder Normal Adjacent (OD04718-03)	11.5
83787 Kidney NAT (OD04338)	2.4	Normal Ovary Res. Gen.	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	2.5	Ovarian Cancer GENPAK 064008	9.1
83789 Kidney NAT (OD04339)	0.0	87492 Ovary Cancer (OD04768-07)	26.6
83790 Kidney Ca, Clear cell type (OD04340)	27.1	87493 Ovary NAT (OD04768-08)	2.5
83791 Kidney NAT (OD04340)	10.7	Normal Stomach GENPAK 061017	7.7
83792 Kidney Ca, Nuclear grade 3 (OD04348)	7.9	Gastric Cancer Clontech 9060358	0.0
83793 Kidney NAT (OD04348)	23.8	NAT Stomach Clontech 9060359	2.7
87474 Kidney Cancer (OD04622-01)	44.8	Gastric Cancer Clontech 9060395	7.9
87475 Kidney NAT (OD04622-03)	3.2	NAT Stomach Clontech 9060394	0.7
85973 Kidney Cancer (OD04450-01)	2.8	Gastric Cancer Clontech 9060397	11.1
85974 Kidney NAT (OD04450-03)	0.0	NAT Stomach Clontech 9060396	2.9
Kidney Cancer Clontech 8120607	0.4	Gastric Cancer GENPAK 064005	11.5

Table 20F. Panel 4D

Tissue Name	Relative	Relative Expression(%)	Relative	Relative
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	Expression (%)			Expression (%)	Expression (%)
	4dtm5337t_ag1609	4Dtm2038f_ag1223	4Dtm2247f_ag1223	4dx4tm4606t_ag2385 a1	4dx4tm4604f_ag2320 a1
93768_Secondary Th1 anti-CD28/anti-CD3	0.0	0.0	0.0	0.0	0.0
93769_Secondary Th2 anti-CD28/anti-CD3	3.4	2.6	0.0	5.3	0.5
93770_Secondary Tr1 anti-CD28/anti-CD3	0.8	0.2	0.0	0.0	0.8
93573_Secondary Th1 resting day 4-6 in IL-2	0.0	0.0	0.0	0.0	0.0
93572_Secondary Th2 resting day 4-6 in IL-2	0.0	0.0	0.6	0.8	0.0
93571_Secondary Tr1 resting day 4-6 in IL-2	0.0	0.6	0.0	0.0	0.0
93568_primary Th1 anti-CD28/anti-CD3	0.0	0.0	0.0	0.0	0.0
93569_primary Th2 anti-CD28/anti-CD3	0.0	0.0	0.0	0.0	0.0
93570_primary Tr1 anti-CD28/anti-CD3	0.0	0.0	0.0	0.0	0.0
93565_primary Th1 resting dy 4-6 in IL-2	0.2	0.8	0.8	0.0	0.0
93566_primary Th2 resting dy 4-6 in IL-2	0.0	0.0	0.0	0.0	0.0
93567_primary Tr1 resting dy 4-6 in IL-2	0.0	0.0	0.0	0.0	0.0
93351_CD45RA CD4 lymphocyte anti-CD28/anti-CD3	0.3	1.3	1.4	0.0	0.6
93352_CD45RO CD4 lymphocyte anti-CD28/anti-CD3	3.2	4.7	3.5	5.1	0.0
93251_CD8 Lymphocytes anti-CD28/anti-CD3	1.2	0.5	0.0	1.2	0.8
93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	1.9	0.0	3.0	0.0	0.6
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	0.0	0.0	0.0	0.0	0.0
93354_CD4_none	0.0	1.4	0.0	0.0	0.7
93252_Secondary Th1/Th2/Tr1 anti-CD95 CH11	0.0	0.0	0.0	0.0	0.0
93103_LAK cells_resting	9.8	6.7	9.8	5.4	3.6
93788_LAK cells_IL-2	2.6	2.0	4.0	1.4	1.7
93787_LAK cells_IL-2+IL-12	3.8	3.1	8.3	3.8	5.2

93789_LAK cells_IL-2+IFN gamma	9.7	11.2	6.0	11.3	10.5
93790_LAK cells_IL-2+IL-18	14.4	9.0	8.9	8.7	4.8
93104_LAK cells_PMA/ionomycin and IL-18	9.0	11.5	18.2	10.5	13.5
93578_NK Cells IL-2 resting	0.0	0.7	0.0	0.0	0.0
93109_Mixed Lymphocyte Reaction Two Way MLR	21.2	13.4	15.7	9.8	15.4
93110_Mixed Lymphocyte Reaction Two Way MLR	6.6	5.6	4.4	1.6	3.2
93111_Mixed Lymphocyte Reaction Two Way MLR	2.4	0.0	1.0	0.0	2.5
93112_Mononuclear Cells (PBMCs) resting	23.7	5.8	9.7	9.6	10.5
93113_Mononuclear Cells (PBMCs) PWM	10.2	9.8	8.5	12.3	12.3
93114_Mononuclear Cells (PBMCs) PHA-L	0.0	0.0	0.7	3.8	1.0
93249_Ramos (B cell) none	0.0	0.0	0.0	0.0	0.0
93250_Ramos (B cell) ionomycin	0.0	0.0	0.0	0.0	0.0
93349_B lymphocytes PWM	0.0	0.0	0.8	1.5	1.7
93350_B lymphocytes_CD40L and IL-4	0.0	0.6	0.0	0.0	0.0
92665_EOL-1 (Eosinophil) dbcAMP differentiated	15.6	10.3	6.7	16.0	3.1
93248_EOL-1 (Eosinophil) dbcAMP/PM Aionomycin	5.7	1.8	4.0	2.6	3.4
93356_Dendritic Cells none	0.0	0.6	0.7	2.9	0.7
93355_Dendritic Cells_LPS 100 ng/ml	2.6	7.2	1.6	1.7	2.8
93775_Dendritic Cells anti-CD40	8.4	4.8	6.4	3.1	4.3
93774_Monocytes_resting	100.0	100.0	100.0	100.0	100.0
93776_Monocytes_LPS 50 ng/ml	3.1	6.6	11.7	4.1	8.0
93581_Macrophages_resting	3.0	1.4	0.2	2.8	1.5
93582_Macrophages_LPS 100 ng/ml	16.5	46.7	52.5	23.1	18.5
93098_HUVEC (Endothelial) none	0.0	0.0	0.0	0.0	0.0

93099_HUVEC (Endothelial) starved	0.0	0.0	0.0	0.0	0.0
93100_HUVEC (Endothelial) IL-1b	19.3	0.0	0.0	0.0	0.0
93779_HUVEC (Endothelial) IFN gamma	2.6	0.9	0.0	0.0	1.5
93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	1.1	0.0	0.0	0.0	0.7
93101_HUVEC (Endothelial)_TNF alpha + IL4	0.0	0.0	0.0	0.0	0.0
93781_HUVEC (Endothelial) IL-11	0.0	0.0	0.0	0.0	0.0
93583_Lung Microvascular Endothelial Cells none	0.0	0.0	0.0	0.0	0.9
93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	0.0	0.0	0.0
92662_Microvascular Dermal endothelium none	0.0	0.0	0.0	0.0	0.3
92663_Microvascular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	0.0	0.0	0.8
93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.0	0.0	0.0	0.0	4.2
93347_Small Airway Epithelium none	0.0	0.0	0.0	0.0	0.0
93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	1.0	0.0	0.0	0.0	0.0
92668_Coronary Artery SMC resting	0.0	0.0	0.0	0.0	0.0
92669_Coronary Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	0.0	0.0	0.0
93107_astrocytes_resting	0.0	0.0	0.0	0.0	0.0
93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0	0.0	0.0	0.0
92666_KU-812 (Basophil) resting	0.0	0.0	0.0	0.0	0.0
92667_KU-812 (Basophil) PMA/ionoycin	2.1	1.4	2.5	1.1	0.0
93579_CCD1106 (Keratinocytes) none	0.0	0.0	0.0	0.0	0.0
93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	0.1	8.2	10.9	1.0	0.8
93791_Liver Cirrhosis	6.0	4.1	5.7	2.5	6.5

93792_Lupus Kidney	0.0	0.0	1.5	0.0	0.0
93577_NCI-H292	0.0	0.0	0.0	0.0	0.0
93358_NCI-H292_IL-4	0.0	0.0	0.0	0.0	0.0
93360_NCI-H292_IL-9	0.0	0.0	0.0	0.0	0.5
93359_NCI-H292_IL-13	0.0	0.0	0.0	0.0	0.0
93357_NCI-H292_IFN gamma	0.3	0.3	0.0	0.0	0.3
93777_HPAEC_-	0.0	0.0	0.0	0.0	0.0
93778_HPAEC_IL-1 beta/TNA alpha	0.0	0.0	0.0	1.4	0.0
93254_Normal Human Lung Fibroblast_none	0.0	0.0	0.0	0.0	0.0
93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0	0.0	0.0	0.0	0.0
93257_Normal Human Lung Fibroblast_IL-4	0.0	0.0	0.0	0.0	0.0
93256_Normal Human Lung Fibroblast_IL-9	0.0	0.0	0.0	0.0	0.0
93255_Normal Human Lung Fibroblast_IL-13	0.0	0.0	0.0	0.0	0.0
93258_Normal Human Lung Fibroblast_IFN gamma	1.2	0.8	1.6	1.2	0.6
93106_Dermal Fibroblasts CCD1070_resting	0.0	0.0	0.0	0.0	0.0
93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	1.1	0.0	0.0	0.0	0.0
93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	0.0	0.0	0.0	0.0	0.0
93772_dermal fibroblast IFN gamma	1.1	0.0	0.0	0.9	2.5
93771_dermal fibroblast IL-4	0.2	0.0	0.0	0.0	0.0
93260_IBD Colitis 2	0.0	0.0	0.0	0.0	0.0
93261_IBD Crohns	0.0	0.5	0.0	0.0	1.5
735010_Colon_normal	0.0	0.0	0.0	0.0	0.0
735019_Lung_none	3.5	0.7	3.1	1.1	0.6
64028-1_Thymus_none	0.0	0.0	0.0	2.3	0.8
64030-1_Kidney_none	0.0	0.7	0.4	2.5	0.7

Table 20G. Panel CNS_neurodegeneration_v1.0

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	tm7002f ag2320_b1_s2		tm7002f ag2320_b1_s2

AD 1 Hippo	17.7	Control (Path) 3 Temporal Ctx	0.0
AD 2 Hippo	0.0	Control (Path) 4 Temporal Ctx	15.2
AD 3 Hippo	3.3	AD 1 Occipital Ctx	10.4
AD 4 Hippo	0.0	AD 2 Occipital Ctx (Missing)	0.0
AD 5 hippo	22.9	AD 3 Occipital Ctx	0.0
AD 6 Hippo	100.0	AD 4 Occipital Ctx	22.2
Control 2 Hippo	25.7	AD 5 Occipital Ctx	25.3
Control 4 Hippo	38.2	AD 6 Occipital Ctx	9.0
Control (Path) 3 Hippo	10.0	Control 1 Occipital Ctx	0.0
AD 1 Temporal Ctx	10.0	Control 2 Occipital Ctx	7.9
AD 2 Temporal Ctx	0.0	Control 3 Occipital Ctx	27.0
AD 3 Temporal Ctx	0.0	Control 4 Occipital Ctx	0.0
AD 4 Temporal Ctx	13.4	Control (Path) 1 Occipital Ctx	26.0
AD 5 Inf Temporal Ctx	41.8	Control (Path) 2 Occipital Ctx	0.0
AD 5 Sup Temporal Ctx	39.1	Control (Path) 3 Occipital Ctx	13.3
AD 6 Inf Temporal Ctx	45.0	Control (Path) 4 Occipital Ctx	13.2
AD 6 Sup Temporal Ctx	87.6	Control 1 Parietal Ctx	51.8
Control 1 Temporal Ctx	0.0	Control 2 Parietal Ctx	39.5
Control 2 Temporal Ctx	32.9	Control 3 Parietal Ctx	0.0
Control 3 Temporal Ctx	0.0	Control (Path) 1 Parietal Ctx	23.0
Control 4 Temporal Ctx	0.0	Control (Path) 2 Parietal Ctx	25.1
Control (Path) 1 Temporal Ctx	18.1	Control (Path) 3 Parietal Ctx	0.0
Control (Path) 2 Temporal Ctx	5.7	Control (Path) 4 Parietal Ctx	13.0

Panel 1.2 Summary Ag1223 Expression of the CG50177-01 gene in this panel is low/undetectable (CT values >35) across all samples (data not shown).

Panel 1.3D Summary: Ag1223/1609/Ag2320 expression of the cg50177-01 gene is highest in and exclusive to a sample derived from gastric cancer cell line nci-n87 (ct=31-34). Apparent expression in other samples is below the threshold of reliability. Thus the expression of this gene could be used to distinguish samples derived from gastric cancer cell lines from other samples. Furthermore, therapeutic modulation of the cg50177-01 gene or its gene product, through the application of small molecule or specific antibodies, could be useful in the treatment of gastric cancer. Ag2385 in this experiment, expression of the cg50177-01 gene is limited to a cluster of samples from lung cancer cell lines. The expression of this gene appears to be highest in a sample derived from a lung cancer cell line (shp-77). In addition there is substantial expression in other samples derived from lung cancer cell lines (5 of 10).

Thus, the expression of the cg50177-01 gene could be used to distinguish lung cancer cell line derived samples from other samples. Moreover, therapeutic modulation of the expression or function of this gene, through the use of small molecule drugs or antibodies, might be of use in the treatment of lung cancer.

5 **Panel 2D Summary Ag1223** The expression profile of the CG50177-01 gene reveals significant levels of expression in a number of tissue samples. Specifically there appear to be clusters of expression in breast, gastric and renal cancers when compared to adjacent normal tissues. This expression profile indicates that therapeutic modulation of the CG50177-01 gene or its protein product could be effective in the treatment of breast, gastric and renal cancers.

10 **Panel 4D Summary Ag1609/Ag1223/Ag2385/Ag2320** Multiple experiments show that expression of the CG50177-01 gene is high in resting monocytes and LPS activated macrophages. This expression profile suggests that the CG50177-01 gene may encode a monocyte differentiation antigen and a macrophage activation antigen. Signalling through this
15 molecule may stimulate differentiation of monocytes to macrophages and macrophages may upregulate this molecule after LPS activation. Therefore, agonistic small molecule therapeutics to the antigen encoded for by this gene could be useful in increasing immune responsiveness during gram negative bacterial infections. Alternatively, antagonistic antibody or small molecule therapeutics could reduce or eliminate inflammation in autoimmune
20 diseases such as asthma/allergy, emphysema, psoriasis, arthritis or other acute or chronic diseases in which activated macrophages play a detrimental role.

25 **Panel CNS_neurodegeneration_v1.0 Summary Ag2320** The CG50177-01 gene encodes a protein with homology to the GPCR family of receptors. This gene is expressed in the brain in this panel, although no clear association of gene expression level with Alzheimer's disease was detected once samples were normalized for RNA loading. Several neurotransmitter receptors are GPCRs, including the dopamine receptor family, the serotonin receptor family, the GABAB receptor, muscarinic acetylcholine receptors, and others; thus this GPCR may represent a novel neurotransmitter receptor. Targeting various neurotransmitter receptors (dopamine, serotonin) has proven to be an
30 effective therapy in psychiatric illnesses such as schizophrenia, bipolar disorder and depression. Furthermore, the cerebral cortex and hippocampus are regions of the brain that are known to play critical roles in Alzheimer's disease, seizure disorders, and in the normal process of memory formation.

Therefore, therapeutic modulation of the CG50177-01 gene or its protein product may be beneficial in the treatment of one or more of these diseases, as may blockade of the receptor coded for by the gene.

5 GPCR7 (also referred to as CG50201-01 and CG50257-01)

Expression of gene CG50201-01 and variant CG50257-01 was assessed using the primer-probe sets Ag2254, Ag1916, Ag2485, and Ag2554, described in Tables 21A and 21B. Results from RTQ-PCR runs are shown in Tables 21C, 21D, 21E and 21F.

Table 21A Probe Name Ag2254/Ag1916 (identical sequences) (SEQ ID NO: 171, 172, 173)

Primers	Sequences	TM	Length	Start Position
Forward	5' - CATCATCTACAGCCTCTGGAAT - 3'	58.3	22	870
Probe	TET-5' - CACTCCGAGCCCTTCTCATTGGG - 3' - TAMRA	70.2	23	908
Reverse	5' - CCTCAGGAGTCACTAGCTGAGA - 3'	58.8	22	938

Table 21B Probe Name Ag2485/Ag2554 (identical sequences) (SEQ ID NO: 174, 175, 176)

Primers	Sequences	TM	Length	Start Position
Forward	5' - CATCATCTACAGCCTCTGGAAT - 3'	58.3	22	867
Probe	FAM-5' - CACTCCGAGCCCTTCTCATTGGG - 3' - TAMRA	70.2	23	905
Reverse	5' - CCTCAGGAGTCACTAGCTGAGA - 3'	58.8	22	935

Table 21C. Panel 1.3D

Tissue Name	Relative Expression (%)	Relative Expression (%)	Relative Expression (%)	Relative Expression (%)
	1.3Dtm2790 f_ag1916	1.3dx4tm54 58f_ag2485 b1	1.3dx4tm54 60f_ag2554 b1	1.3dx4tm56 22t_ag2254 a1
Liver adenocarcinoma	10.9	39.3	28.0	13.1
Pancreas	19.1	0.0	0.0	4.6
Pancreatic ca. CAPAN 2	0.0	0.0	4.3	0.0
Adrenal gland	10.6	21.0	4.0	0.0
Thyroid	14.9	41.6	14.9	7.0
Salivary gland	7.6	0.0	0.0	5.4
Pituitary gland	74.7	28.8	0.0	21.7
Brain (fetal)	3.2	18.0	6.5	5.6
Brain (whole)	36.3	74.3	41.2	17.8

Brain (amygdala)	6.1	100.0	10.1	44.1
Brain (cerebellum)	0.0	66.7	11.6	26.5
Brain (hippocampus)	36.1	76.8	23.5	13.4
Brain (substantia nigra)	9.4	28.4	19.5	13.0
Brain (thalamus)	30.6	13.0	33.7	20.3
Cerebral Cortex	43.2	13.9	4.8	0.0
Spinal cord	18.7	62.5	27.9	20.9
CNS ca. (glio/astro) U87-MG	17.6	33.7	0.0	6.8
CNS ca. (glio/astro) U-118-MG	18.7	0.0	4.7	0.0
CNS ca. (astro) SW1783	9.0	27.0	9.9	13.2
CNS ca.* (neuro; met) SK-N-AS	5.2	0.0	3.3	0.0
CNS ca. (astro) SF-539	12.8	13.6	0.0	6.0
CNS ca. (astro) SNB-75	21.0	0.0	1.2	0.0
CNS ca. (glio) SNB-19	7.7	26.8	0.0	6.0
CNS ca. (glio) U251	12.1	22.4	11.9	0.0
CNS ca. (glio) SF-295	14.2	19.8	41.0	6.2
Heart (fetal)	0.0	0.0	0.0	0.0
Heart	11.7	14.5	14.8	0.0
Fetal Skeletal	94.6	7.6	13.5	0.0
Skeletal muscle	0.0	0.0	0.0	0.0
Bone marrow	15.3	68.5	16.2	11.1
Thymus	57.8	0.0	0.0	17.5
Spleen	29.7	33.4	4.2	100.0
Lymph node	16.2	40.5	21.7	4.6
Colorectal	58.6	78.2	7.2	5.7
Stomach	27.4	34.4	13.8	19.0
Small intestine	9.9	32.8	9.6	0.0
Colon ca. SW480	12.3	0.0	0.0	0.0
Colon ca.* (SW480 met)SW620	0.0	0.0	0.0	0.0
Colon ca. HT29	0.0	0.0	0.0	0.0
Colon ca. HCT-116	0.0	7.4	6.9	0.0
Colon ca. CaCo-2	0.0	0.0	0.0	3.6
83219 CC Well to Mod Diff (ODO3866)	0.0	8.3	0.0	0.0
Colon ca. HCC-2998	0.0	0.0	0.0	0.0
Gastric ca.* (liver met) NCI-N87	11.9	14.3	6.3	1.3
Bladder	0.0	59.6	0.0	2.9
Trachea	22.8	24.4	5.6	4.4
Kidney	0.0	6.1	0.0	0.0
Kidney (fetal)	8.6	0.0	10.1	0.0
Renal ca. 786-0	0.0	0.0	2.7	9.0
Renal ca. A498	37.4	41.9	100.0	0.0
Renal ca. RXF 393	0.0	10.6	10.1	0.0

Renal ca. ACHN	5.5	0.0	0.0	1.4
Renal ca. UO-31	3.6	10.6	0.0	1.7
Renal ca. TK-10	0.0	0.0	3.2	0.0
Liver	9.0	7.4	8.8	0.0
Liver (fetal)	12.2	68.4	11.8	5.2
Liver ca. (hepatoblast) HepG2	6.6	22.4	3.9	0.0
Lung	17.7	7.8	14.5	1.9
Lung (fetal)	18.4	13.9	0.0	3.7
Lung ca. (small cell) LX-1	0.0	0.0	0.0	7.5
Lung ca. (small cell) NCI-H69	0.0	11.9	0.0	0.0
Lung ca. (s.cell var.) SHP-77	19.8	0.0	10.6	10.5
Lung ca. (large cell)NCI-H460	10.2	0.0	0.8	0.0
Lung ca. (non-sm. cell) A549	9.5	0.0	16.1	4.6
Lung ca. (non-s.cell) NCI-H23	5.8	13.0	9.6	5.9
Lung ca (non-s.cell) HOP-62	6.4	0.0	0.0	0.0
Lung ca. (non-s.cl) NCI-H522	0.0	0.0	0.0	0.0
Lung ca. (squam.) SW 900	10.3	0.0	17.5	0.0
Lung ca. (squam.) NCI-H596	13.2	13.0	0.0	0.0
Mammary gland	28.3	40.8	18.1	5.6
Breast ca.* (pl. effusion) MCF-7	0.0	25.2	0.0	4.6
Breast ca.* (pl.ef) MDA-MB-231	4.9	0.0	7.8	0.0
Breast ca.* (pl. effusion) T47D	6.2	0.0	7.6	0.0
Breast ca. BT-549	0.0	11.8	9.2	0.0
Breast ca. MDA-N	3.3	0.0	4.8	0.0
Ovary	4.0	0.0	0.0	0.0
Ovarian ca. OVCAR-3	5.7	0.0	0.0	4.1
Ovarian ca. OVCAR-4	0.0	48.4	0.0	0.0
Ovarian ca. OVCAR-5	0.0	0.0	0.0	0.0
Ovarian ca. OVCAR-8	30.6	0.0	9.2	0.0
Ovarian ca. IGROV-1	0.0	13.5	9.9	0.0
Ovarian ca.* (ascites) SK-OV-3	17.4	0.0	6.4	18.4
Uterus	5.1	17.7	23.4	26.8
Placenta	47.3	30.4	40.7	27.5
Prostate	29.1	38.4	11.9	13.6
Prostate ca.* (bone met)PC-3	0.0	13.2	0.0	0.0
Testis	100.0	72.5	14.7	4.7
Melanoma Hs688(A).T	13.2	0.0	0.0	0.0
Melanoma* (met) Hs688(B).T	10.2	26.2	27.4	0.0
Melanoma UACC-62	0.0	0.0	0.0	0.0
Melanoma M14	0.0	0.0	5.1	0.0
Melanoma LOX IMVI	0.0	0.0	0.0	0.0
Melanoma* (met) SK-MEL-5	0.0	0.0	0.0	0.0
Adipose	0.0	0.0	0.0	0.0

Table 21D. Panel 2.2

Tissue Name	Relative Expression(%) 2.2x4tm6426t ag2254_b2	Tissue Name	Relative Expression(%) 2.2x4tm6426t ag2254_b2
Normal Colon GENPAK 061003	0.9	83793 Kidney NAT (OD04348)	0.1
97759 Colon cancer (OD06064)	0.0	98938 Kidney malignant cancer (OD06204B)	0.0
97760 Colon cancer NAT (OD06064)	0.1	98939 Kidney normal adjacent tissue (OD06204E)	0.0
97778 Colon cancer (OD06159)	0.5	85973 Kidney Cancer (OD04450-01)	0.0
97779 Colon cancer NAT (OD06159)	100.0	85974 Kidney NAT (OD04450-03)	0.0
98861 Colon cancer (OD06297-04)	0.0	Kidney Cancer Clontech 8120613	0.0
98862 Colon cancer NAT (OD06297-015)	0.0	Kidney NAT Clontech 8120614	0.0
83237 CC Gr.2 ascend colon (ODO3921)	0.0	Kidney Cancer Clontech 9010320	0.0
83238 CC NAT (ODO3921)	0.0	Kidney NAT Clontech 9010321	0.0
97766 Colon cancer metastasis (OD06104)	0.6	Kidney Cancer Clontech 8120607	0.3
97767 Lung NAT (OD06104)	0.0	Kidney NAT Clontech 8120608	0.0
87472 Colon mets to lung (OD04451-01)	0.0	Normal Uterus GENPAK 061018	0.0
87473 Lung NAT (OD04451-02)	0.4	Uterus Cancer GENPAK 064011	0.0
Normal Prostate Clontech A+ 6546-1 (8090438)	0.3	Normal Thyroid Clontech A+ 6570-1 (7080817)	0.1
84140 Prostate Cancer (OD04410)	0.0	Thyroid Cancer GENPAK 064010	0.1
84141 Prostate NAT (OD04410)	0.0	Thyroid Cancer INVITROGEN A302152	0.0
Normal Ovary Res. Gen.	0.2	Thyroid NAT INVITROGEN A302153	0.0
98863 Ovarian cancer (OD06283-03)	0.0	Normal Breast GENPAK 061019	0.0
98865 Ovarian cancer NAT/fallopian tube (OD06283-07)	0.0	84877 Breast Cancer (OD04566)	0.2
Ovarian Cancer GENPAK 064008	0.1	Breast Cancer Res. Gen. 1024	0.1
97773 Ovarian cancer (OD06145)	0.0	85975 Breast Cancer (OD04590-01)	0.2
97775 Ovarian cancer NAT (OD06145)	0.1	85976 Breast Cancer Mets (OD04590-03)	0.1

98853 Ovarian cancer (OD06455-03)	0.0	87070 Breast Cancer Metastasis (OD04655-05)	0.4
98854 Ovarian NAT (OD06455-07) Fallopian tube	0.0	GENPAK Breast Cancer 064006	0.1
Normal Lung GENPAK 061010	0.2	Breast Cancer Clontech 9100266	0.5
92337 Invasive poor diff. lung adeno (ODO4945-01)	0.0	Breast NAT Clontech 9100265	0.0
92338 Lung NAT (ODO4945- 03)	0.0	Breast Cancer INVITROGEN A209073	0.1
84136 Lung Malignant Cancer (OD03126)	0.3	Breast NAT INVITROGEN A2090734	0.1
84137 Lung NAT (OD03126)	0.0	97763 Breast cancer (OD06083)	0.1
90372 Lung Cancer (OD05014A)	0.2	97764 Breast cancer node metastasis (OD06083)	0.0
90373 Lung NAT (OD05014B)	0.1	Normal Liver GENPAK 061009	0.0
97761 Lung cancer (OD06081)	0.0	Liver Cancer Research Genetics RNA 1026	0.0
97762 Lung cancer NAT (OD06081)	0.1	Liver Cancer Research Genetics RNA 1025	0.0
85950 Lung Cancer (OD04237- 01)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6004- T	0.1
85970 Lung NAT (OD04237- 02)	0.1	Paired Liver Tissue Research Genetics RNA 6004-N	0.1
83255 Ocular Mel Met to Liver (ODO4310)	0.1	Paired Liver Cancer Tissue Research Genetics RNA 6005- T	0.1
83256 Liver NAT (ODO4310)	0.1	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
84139 Melanoma Mets to Lung (OD04321)	0.0	Liver Cancer GENPAK 064003	0.0
84138 Lung NAT (OD04321)	0.0	Normal Bladder GENPAK 061001	0.1
Normal Kidney GENPAK 061008	0.0	Bladder Cancer Research Genetics RNA 1023	0.2
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	Bladder Cancer INVITROGEN A302173	0.2
83787 Kidney NAT (OD04338)	0.0	Normal Stomach GENPAK 061017	0.3
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.1	Gastric Cancer Clontech 9060397	0.0
83789 Kidney NAT (OD04339)	0.0	NAT Stomach Clontech 9060396	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	Gastric Cancer Clontech 9060395	0.2
83791 Kidney NAT (OD04340)	0.4	NAT Stomach Clontech 9060394	0.0

83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.1	Gastric Cancer GENPAK 064005	0.2
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Table 21E. Panel 4D

Tissue Name	Relative Expression(%) 4dx4tm4409f_ ag1916_b2	Relative Expression(%) 4dx4tm5035f_ ag2554_a1	Relative Expression(%) 4dx4tm5033f_ ag2485_b2	Relative Expression(%) 4dx4tm4908t_ ag2254_b1
93768_Secondary Th1_anti- CD28/anti-CD3	33.4	79.9	19.1	36.7
93769_Secondary Th2_anti- CD28/anti-CD3	16.1	57.3	9.9	41.9
93770_Secondary Tr1_anti- CD28/anti-CD3	26.9	38.1	39.6	12.4
93573_Secondary Th1_resting day 4-6 in IL-2	23.1	21.4	18.7	21.5
93572_Secondary Th2_resting day 4-6 in IL-2	50.7	23.6	11.8	58.7
93571_Secondary Tr1_resting day 4-6 in IL-2	54.3	20.9	39.0	72.4
93568_primary Th1_anti- CD28/anti-CD3	20.8	24.2	3.8	17.3
93569_primary Th2_anti- CD28/anti-CD3	15.8	5.2	10.7	22.6
93570_primary Tr1_anti- CD28/anti-CD3	0.0	25.8	9.5	11.1
93565_primary Th1_resting dy 4-6 in IL-2	48.9	38.9	61.9	70.6
93566_primary Th2_resting dy 4-6 in IL-2	27.7	26.4	44.7	25.1
93567_primary Tr1_resting dy 4-6 in IL-2	16.3	30.4	7.9	72.6
93351_CD45RA CD4 lymphocyte_anti-CD28/anti- CD3	4.8	3.1	18.5	31.5
93352_CD45RO CD4 lymphocyte_anti-CD28/anti- CD3	16.4	34.2	18.1	45.1
93251_CD8 Lymphocytes_anti- CD28/anti-CD3	16.6	38.6	11.8	17.9
93353_chronic CD8 Lymphocytes 2ry_resting dy 4- 6 in IL-2	10.6	25.7	19.1	14.7
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	3.8	28.5	13.9	19.6
93354_CD4_none	4.1	12.3	9.3	6.0
93252_Secondary	97.6	83.4	100.0	68.8

Th1/Th2/Tr1_anti-CD95 CH11				
93103_LAK cells_resting	24.2	36.1	9.5	31.3
93788_LAK cells_IL-2	43.4	20.2	38.9	45.6
93787_LAK cells_IL-2+IL-12	15.3	28.5	13.9	17.0
93789_LAK cells_IL-2+IFN gamma	51.1	38.3	70.9	24.2
93790_LAK cells_IL-2+ IL-18	52.1	36.2	44.1	19.3
93104_LAK cells_PMA/ionomycin and IL- 18	0.0	0.0	8.2	0.0
93578_NK Cells IL-2_resting	20.6	13.6	5.6	35.7
93109_Mixed Lymphocyte Reaction_Two Way MLR	13.5	19.9	27.9	49.3
93110_Mixed Lymphocyte Reaction_Two Way MLR	0.0	0.0	4.6	5.8
93111_Mixed Lymphocyte Reaction_Two Way MLR	16.7	9.2	4.0	25.6
93112_Mononuclear Cells (PBMCs)_resting	0.0	3.8	14.3	2.9
93113_Mononuclear Cells (PBMCs)_PWM	24.1	55.2	11.6	42.3
93114_Mononuclear Cells (PBMCs)_PHA-L	28.2	2.9	8.8	34.6
93249_Ramos (B cell)_none	0.0	18.2	9.8	0.0
93250_Ramos (B cell)_ionomycin	5.3	15.9	8.2	5.8
93349_B lymphocytes PWM	11.7	11.8	16.1	47.7
93350_B lymphocytes_CD40L and IL-4	51.9	36.5	27.3	35.5
92665_EOL-1 (Eosinophil)_dbcAMP differentiated	13.3	15.4	31.4	0.0
93248_EOL-1 (Eosinophil)_dbcAMP/PMAion omycin	6.1	5.2	9.0	0.0
93356_Dendritic Cells_none	11.5	20.4	30.1	0.0
93355_Dendritic Cells_LPS 100 ng/ml	11.3	5.8	15.9	14.6
93775_Dendritic Cells_anti- CD40	22.3	17.7	10.1	13.2
93774_Monocytes_resting	12.7	14.3	7.2	8.2
93776_Monocytes_LPS 50 ng/ml	25.1	17.0	8.4	30.1
93581_Macrophages_resting	0.0	11.1	5.9	6.2
93582_Macrophages_LPS 100 ng/ml	21.3	22.2	2.6	22.2
93098_HUVEC (Endothelial)_none	54.4	27.0	19.6	50.7

93099_HUVEC (Endothelial) starved	76.2	55.5	17.5	97.7
93100_HUVEC (Endothelial) IL-1b	12.4	3.4	0.0	6.9
93779_HUVEC (Endothelial) IFN gamma	100.0	87.7	62.1	92.8
93102_HUVEC (Endothelial) TNF alpha + IFN gamma	13.8	0.0	22.6	16.7
93101_HUVEC (Endothelial) TNF alpha + IL4	10.4	9.0	10.4	0.0
93781_HUVEC (Endothelial) IL-11	35.9	39.9	21.0	70.7
93583_Lung Microvascular Endothelial Cells None	29.4	27.2	32.3	100.0
93584_Lung Microvascular Endothelial Cells TNFa (4 ng/ml) and IL1b (1 ng/ml)	25.6	52.2	13.5	77.5
92662_Microvascular Dermal endothelium None	30.7	52.6	55.9	88.7
92663_Microvascular Dermal endothelium TNFa (4 ng/ml) and IL1b (1 ng/ml)	26.7	30.3	5.9	46.3
93773_Bronchial epithelium TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.0	7.5	19.8	17.1
93347_Small Airway Epithelium None	0.0	9.2	8.2	17.4
93348_Small Airway Epithelium TNFa (4 ng/ml) and IL1b (1 ng/ml)	27.0	18.5	21.8	65.5
92668_Coronary Artery SMC resting	8.1	9.7	5.8	21.7
92669_Coronary Artery SMC TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	6.7	0.0	5.8
93107_astrocytes resting	5.1	0.0	4.4	4.9
93108_astrocytes TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	5.7	0.0	13.5
92666_KU-812 (Basophil) resting	27.8	12.0	6.5	25.1
92667_KU-812 (Basophil) PMA/ionoycin	28.9	59.1	20.5	21.8
93579_CCD1106 (Keratinocytes) None	2.4	0.0	4.4	6.0
93580_CCD1106 (Keratinocytes) TNFa and IFNg **	0.0	0.0	4.2	0.0
93791_Liver Cirrhosis	8.5	34.8	30.7	33.4

93792_Lupus Kidney	0.0	10.9	0.0	5.0
93577_NCI-H292	24.9	29.6	13.8	21.9
93358_NCI-H292_IL-4	5.6	20.9	4.2	24.9
93360_NCI-H292_IL-9	60.8	0.0	8.7	10.5
93359_NCI-H292_IL-13	0.0	7.1	17.7	5.2
93357_NCI-H292_IFN gamma	20.2	13.2	9.2	2.8
93777_HPAEC -	50.6	100.0	47.1	46.0
93778_HPAEC_IL-1 beta/TNA alpha	44.5	16.4	10.7	21.0
93254_Normal Human Lung Fibroblast_none	19.9	7.6	18.7	6.1
93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	11.5	0.0	10.2	20.9
93257_Normal Human Lung Fibroblast_IL-4	25.9	10.2	11.0	15.7
93256_Normal Human Lung Fibroblast_IL-9	5.0	23.5	6.6	24.0
93255_Normal Human Lung Fibroblast_IL-13	9.7	17.2	15.6	17.3
93258_Normal Human Lung Fibroblast_IFN gamma	19.3	8.9	8.7	18.0
93106_Dermal Fibroblasts CCD1070_resting	3.8	31.8	7.9	36.9
93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	50.8	29.8	47.1	62.4
93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	5.6	11.7	2.1	28.8
93772_dermal fibroblast_IFN gamma	7.0	19.1	11.1	22.7
93771_dermal fibroblast_IL-4	21.5	9.1	15.9	15.5
93260_IBD Colitis 2	5.0	4.9	2.4	0.0
93261_IBD Crohns	0.0	0.0	5.2	5.6
735010_Colon_normal	11.8	18.6	26.4	22.5
735019_Lung_none	29.2	23.1	10.1	44.9
64028-1_Thymus_none	58.0	42.0	36.7	49.7
64030-1_Kidney_none	56.0	83.5	32.6	55.9

Table 21F. Panel CNS_neurodegeneration_v1.0

Tissue Name	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)
	tm7041f_ag2485_a2	tm7007t_ag2254_a2_s1	tm6942f_ag2554_a2
AD 1 Hippo	6.8	18.5	0.0
AD 2 Hippo	13.7	7.0	16.7

AD 3 Hippo	6.1	0.0	7.7
AD 4 Hippo	14.3	17.7	13.0
AD 5 Hippo	39.9	55.3	36.0
AD 6 Hippo	34.7	99.0	42.2
Control 2 Hippo	12.7	11.3	14.8
Control 4 Hippo	3.1	16.3	20.1
Control (Path) 3 Hippo	5.6	4.8	1.7
AD 1 Temporal Ctx	2.5	7.9	21.6
AD 2 Temporal Ctx	41.0	41.7	59.0
AD 3 Temporal Ctx	0.0	12.1	0.0
AD 4 Temporal Ctx	30.4	62.6	23.9
AD 5 Inf Temporal Ctx	100.0	82.6	88.9
AD 5 Sup Temporal Ctx	64.0	17.2	35.6
AD 6 Inf Temporal Ctx	23.2	82.7	84.1
AD 6 Sup Temporal Ctx	52.2	56.3	87.2
Control 1 Temporal Ctx	2.5	0.0	0.0
Control 2 Temporal Ctx	10.3	31.2	19.7
Control 3 Temporal Ctx	6.0	5.5	0.0
Control 3 Temporal Ctx	27.0	7.1	12.0
Control (Path) 1 Temporal Ctx	50.6	59.4	60.8
Control (Path) 2 Temporal Ctx	22.0	14.2	29.6
Control (Path) 3 Temporal Ctx	7.5	5.4	0.0
Control (Path) 4 Temporal Ctx	22.3	42.6	64.0
AD 1 Occipital Ctx	11.9	6.7	18.6
AD 2 Occipital Ctx (Missing)	0.0	0.0	0.0
AD 3 Occipital Ctx	57.6	10.9	0.0
AD 4 Occipital Ctx	47.1	44.9	26.4
AD 5 Occipital Ctx	16.0	25.8	9.3
AD 6 Occipital Ctx	4.0	37.8	42.7
Control 1 Occipital Ctx	0.0	0.0	0.0
Control 2 Occipital Ctx	28.8	37.7	69.2
Control 3 Occipital Ctx	14.6	5.8	16.3
Control 4 Occipital Ctx	5.7	5.1	4.2
Control (Path) 1 Occipital Ctx	79.0	39.2	100.0
Control (Path) 2 Occipital Ctx	17.8	18.3	0.0
Control (Path) 3 Occipital Ctx	0.0	0.0	0.0
Control (Path) 4 Occipital Ctx	29.6	6.6	10.2
Control 1 Parietal Ctx	10.7	7.7	0.0
Control 2 Parietal Ctx	38.9	80.9	84.2
Control 3 Parietal Ctx	2.6	39.7	11.1
Control (Path) 1 Parietal Ctx	37.1	51.8	54.5
Control (Path) 2 Parietal Ctx	15.4	27.2	26.8

Control (Path) 3 Parietal Ctx	0.0	0.0	6.2
Control (Path) 4 Parietal Ctx	53.6	100.0	46.9

Panel 1.3D Summary Four experiments using two different probe/primer sets show somewhat disparate results, most likely due to the low level of CG50201-01 gene expression in this panel.

Ag2554 The CG50201-01 gene is most highly expressed in a sample derived from renal cancer cell line A498 (CT=32.7). Thus, the expression of this gene could be used to distinguish samples derived from the renal cancer cell line from other samples. Moreover, therapeutic modulation of the expression or function of the protein encoded by the CG50201-01 gene, through the use of small molecule drugs or antibodies, may be useful in the treatment of renal cancer. Ag1916 In this experiment, expression of the CG50201-01 gene is limited to testis (CT=34.6). Ag2485 In this experiment, low but significant expression of the CG50201-01 gene is limited to amygdala (CT=34.6) and hippocampus (CT=34.9). This result suggests a potential role for the CG50201-01 gene in central nervous system function and is consistent with what is seen in CNS_neurodegeneration_panel_v1.0. Ag2254 In this experiment, low but significant expression of the CG50201-01 gene is limited to amygdala (CT=34.4) and spleen (CT=33.2).

Panel 2.2 Summary Ag2254 The CG50201-01 gene is most highly expressed in a sample from normal colon tissue adjacent to a tumor (CT=27). Thus, the expression of this gene could be used to distinguish samples derived from normal colon tissue adjacent to colon cancers. Since there appears to be differential expression of the CG50201-01 gene between normal colon tissue and colon cancer, therapeutic upregulation of the protein encoded by this gene may be effective in the treatment of colon cancer.

Panel 4D Summary Ag1916/Ag2554/Ag2485/Ag2254 Four experiments using two different probe/primer sets show somewhat disparate results, most likely due to the low level of CG50201-01 gene expression in this panel. This gene is reproducibly found at background levels or slightly higher in most of the samples on this panel.

Panel CNS_neurodegeneration_v1.0 Summary Ag2554/Ag2485/Ag2254 In this panel, the CG50201-01 gene is expressed more highly in the hippocampus of some patients with Alzheimer's disease than in control brain without amyloid plaques, which are diagnostic and potentially causative of Alzheimer's disease. GPCRs are readily targetable with drugs, and regulate many specific brain processes, including signaling processes that are currently the target of FDA-approved pharmaceuticals

that treat Alzheimer's disease, such as the cholinergic system. The major mechanisms proposed for amyloid beta-induced cytotoxicity involve the loss of Ca²⁺ homeostasis and the generation of reactive oxygen species (ROS). The changes in Ca²⁺ homeostasis could be the result of changes in G-protein-driven releases of second messengers. Thus, targeting this class of molecule can have therapeutic potential in Alzheimer's disease treatment. In particular, the increased expression of the CG50201-01 gene in brains affected by Alzheimer's indicates potential therapeutic value to drugs that target this GPCR.

GPCR8 (also referred to as CG50193-01):

Expression of gene CG50193-01 was assessed using the primer-probe sets Ag2537, Ag2479, Ag2201, and Ag2433 described in Tables 22A, 22B, and 22C. Results from RTQ-PCR runs are shown in Tables 22D, 22E, 22F, 22G, 22H, and 22I.

Table 22A Probe Name Ag2537/2479 (SEQ ID NO: 177, 178, 179)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GAGGAGAATGCTGCTGATGTAC-3'	59	22	372
Probe	TET-5'-TGGTCTCATACACAGTGATGTCGCCA-3'-TAMRA	69.9	26	398
Reverse	5'-CCAGCTGTTGTGAAGTTGGTAT-3'	59.2	22	425

Table 22B. Probe Name Ag2201 (SEQ ID NO: 180, 181, 182)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GGCAGAAGAATCAGACCTCTCT-3'	59.1	22	96
Probe	FAM-5'-ACTTCATCCTTGAGGGCTCTTCGAT-3'-TAMRA	69.1	26	123
Reverse	5'-GAGAAAAGGAAAAGGTGGGTAA-3'	58.7	22	156

Table 22C. Probe Name Ag2433 (SEQ ID NO: 183, 184, 185)

Primers	Sequences	TM	Length	Start Position
Forward	5'-CTCATCTGGGAGCAAGAGAA-3'	58.1	20	690
Probe	TET-5'-ACTTGTGGCTCCACCTCACGGT-3'-TAMRA	70.3	23	721
Reverse	5'-AGGCACCAAACCAAGAGA-3'	58.3	19	748

Table 22D. Panel 1.3D

Tissue Name	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)
	1.3dx4tm5400t ag2537 a2	1.3dx4tm5626f ag2201 b2	1.3dx4tm5457f ag2479 b1
Liver adenocarcinoma	6.5	8.3	11.9
Pancreas	10.1	0.0	31.2
Pancreatic ca. CAPAN 2	0.0	0.0	0.0
Adrenal gland	11.6	8.6	7.2
Thyroid	2.3	0.0	5.4
Salivary gland	11.6	0.0	5.9
Pituitary gland	7.0	16.9	62.7
Brain (fetal)	11.4	8.4	0.0
Brain (whole)	0.0	4.7	41.0
Brain (amygdala)	12.6	16.1	46.3
Brain (cerebellum)	51.0	56.2	38.9
Brain (hippocampus)	25.8	5.6	31.7
Brain (substantia nigra)	10.9	0.0	21.8
Brain (thalamus)	0.0	23.8	13.2
Cerebral Cortex	9.4	3.2	12.4
Spinal cord	0.0	0.0	21.2
CNS ca. (glio/astro) U87-MG	29.3	35.3	24.9
CNS ca. (glio/astro) U-118-MG	20.2	17.6	18.9
CNS ca. (astro) SW1783	0.0	0.0	37.9
CNS ca.* (neuro; met) SK-N-AS	7.5	8.9	9.8
CNS ca. (astro) SF-539	0.0	100.0	71.8
CNS ca. (astro) SNB-75	12.1	0.0	18.1
CNS ca. (glio) SNB-19	8.6	9.0	8.7
CNS ca. (glio) U251	91.2	18.1	37.0
CNS ca. (glio) SF-295	100.0	45.0	83.7
Heart (fetal)	0.0	12.1	10.0
Heart	0.0	0.0	0.0
Fetal Skeletal	0.0	8.9	0.0
Skeletal muscle	0.0	0.0	0.0
Bone marrow	0.0	0.0	0.0
Thymus	11.3	22.0	0.0
Spleen	11.9	0.0	100.0
Lymph node	23.0	12.7	0.0
Colorectal	46.8	38.7	20.6
Stomach	37.8	0.0	0.0
Small intestine	23.8	9.6	21.1

Colon ca. SW480	0.0	0.0	0.0
Colon ca.* (SW480 met)SW620	0.0	8.9	10.4
Colon ca. HT29	0.0	0.0	0.0
Colon ca. HCT-116	8.0	0.0	17.2
Colon ca. CaCo-2	11.6	0.0	23.0
83219 CC Well to Mod Diff (ODO3866)	12.5	6.2	0.0
Colon ca. HCC-2998	0.0	7.7	13.1
Gastric ca.* (liver met) NCI-N87	0.0	0.0	13.9
Bladder	0.0	21.8	5.0
Trachea	0.0	0.0	13.2
Kidney	0.0	0.0	11.9
Kidney (fetal)	0.0	9.4	24.5
Renal ca. 786-0	10.9	8.5	22.4
Renal ca. A498	9.4	23.6	44.5
Renal ca. RXF 393	0.0	0.0	22.5
Renal ca. ACHN	11.1	8.7	16.7
Renal ca. UO-31	0.0	0.0	0.0
Renal ca. TK-10	0.0	16.4	12.3
Liver	0.0	5.3	0.0
Liver (fetal)	0.0	0.0	0.0
Liver ca. (hepatoblast) HepG2	0.0	0.0	1.6
Lung	0.0	9.7	0.0
Lung (fetal)	14.4	7.7	0.0
Lung ca. (small cell) LX-1	26.3	8.7	36.8
Lung ca. (small cell) NCI-H69	0.0	0.0	11.0
Lung ca. (s.cell var.) SHP-77	0.0	9.1	11.3
Lung ca. (large cell)NCI-H460	0.0	0.0	18.2
Lung ca. (non-sm. cell) A549	0.0	0.0	9.5
Lung ca. (non-s.cell) NCI-H23	24.0	2.6	23.9
Lung ca (non-s.cell) HOP-62	48.6	7.9	37.4
Lung ca. (non-s.cl) NCI-H522	9.1	6.1	0.0
Lung ca. (squam.) SW 900	12.4	10.3	0.0
Lung ca. (squam.) NCI-H596	12.4	15.3	0.0
Mammary gland	0.0	0.0	0.0
Breast ca.* (pl. effusion) MCF-7	0.0	0.0	0.0
Breast ca.* (pl.ef) MDA-MB-231	40.7	0.0	19.0
Breast ca.* (pl. effusion) T47D	0.0	0.0	0.0
Breast ca. BT-549	23.1	0.0	11.4
Breast ca. MDA-N	0.0	0.0	0.0
Ovary	0.0	0.0	0.0
Ovarian ca. OVCAR-3	0.0	0.0	0.0
Ovarian ca. OVCAR-4	0.0	17.4	0.0

Ovarian ca. OVCAR-5	44.2	55.1	32.2
Ovarian ca. OVCAR-8	0.0	0.0	26.1
Ovarian ca. IGROV-1	7.2	0.0	0.0
Ovarian ca.* (ascites) SK-OV-3	32.5	0.0	0.0
Uterus	12.6	0.0	0.0
Placenta	12.3	0.0	19.0
Prostate	0.0	0.0	10.4
Prostate ca.* (bone met)PC-3	23.0	9.8	7.2
Testis	1.8	0.0	10.4
Melanoma Hs688(A).T	12.5	0.0	19.5
Melanoma* (met) Hs688(B).T	11.5	10.6	24.2
Melanoma UACC-62	12.9	0.0	55.7
Melanoma M14	0.0	0.0	0.0
Melanoma LOX IMVI	0.0	0.0	0.0
Melanoma* (met) SK-MEL-5	0.0	0.0	0.0
Adipose	11.6	0.0	0.0

Table 22E. Panel 2.2

Tissue Name	Relative Expression(%) 2.2x4tn6480f_ ag2479 b1	Relative Expression(%) 2.2x4tm6462_ ag2433 a2
Normal Colon GENPAK 061003	29.9	6.4
97759 Colon cancer (OD06064)	0.0	0.0
97760 Colon cancer NAT (OD06064)	5.9	0.0
97778 Colon cancer (OD06159)	0.0	0.0
97779 Colon cancer NAT (OD06159)	9.1	16.7
98861 Colon cancer (OD06297-04)	0.0	0.0
98862 Colon cancer NAT (OD06297-015)	9.5	9.3
83237 CC Gr.2 ascend colon (ODO3921)	6.3	0.0
83238 CC NAT (ODO3921)	0.0	0.0
97766 Colon cancer metastasis (OD06104)	0.0	0.0
97767 Lung NAT (OD06104)	0.0	0.0
87472 Colon mets to lung (OD04451-01)	17	15.6
87473 Lung NAT (OD04451-02)	34.8	27.3
Normal Prostate Clontech A+ 6546-1 (8090438)	19.7	6.3
84140 Prostate Cancer (OD04410)	18.9	0.0
84141 Prostate NAT (OD04410)	9.3	6.7
Normal Ovary Res. Gen.	11.8	0.0
98863 Ovarian cancer (OD06283-03)	16.4	8.0
98865 Ovarian cancer NAT/fallopian tube (OD06283-07)	10.4	16.4

Ovarian Cancer GENPAK 064008	0.0	31.1
97773 Ovarian cancer (OD06145)	0.0	0.0
97775 Ovarian cancer NAT (OD06145)	58.7	45.9
98853 Ovarian cancer (OD06455-03)	32.3	0.0
98854 Ovarian NAT (OD06455-07) Fallopian tube	6.4	11.7
Normal Lung GENPAK 061010	0.0	13.0
92337 Invasive poor diff. lung adeno (ODO4945-01)	0.0	0.0
92338 Lung NAT (ODO4945-03)	22.5	7.5
84136 Lung Malignant Cancer (OD03126)	0.0	0.0
84137 Lung NAT (OD03126)	0.0	22.7
90372 Lung Cancer (OD05014A)	20.3	6.5
90373 Lung NAT (OD05014B)	0.0	18.2
97761 Lung cancer (OD06081)	8.8	14.2
97762 Lung cancer NAT (OD06081)	15.1	0.0
85950 Lung Cancer (OD04237-01)	8.6	11.0
85970 Lung NAT (OD04237-02)	18.9	22.2
83255 Ocular Mel Met to Liver (ODO4310)	9.2	12.7
83256 Liver NAT (ODO4310)	5.5	0.0
84139 Melanoma Mets to Lung (OD04321)	0.0	0.0
84138 Lung NAT (OD04321)	0.0	0.0
Normal Kidney GENPAK 061008	15.8	7.4
83786 Kidney Ca, Nuclear grade 2 (OD04338)	8.5	24.3
83787 Kidney NAT (OD04338)	0.0	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	39.8	0.0
83789 Kidney NAT (OD04339)	9.9	11.9
83790 Kidney Ca, Clear cell type (OD04340)	0.0	0.0
83791 Kidney NAT (OD04340)	6.0	7.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0
83793 Kidney NAT (OD04348)	67.2	26.1
98938 Kidney malignant cancer (OD06204B)	10.4	10.8
98939 Kidney normal adjacent tissue (OD06204E)	14.7	0.0
85973 Kidney Cancer (OD04450-01)	20.1	2.2
85974 Kidney NAT (OD04450-03)	14.6	7.6
Kidney Cancer Clontech 8120613	0.0	0.0
Kidney NAT Clontech 8120614	0.0	6.7
Kidney Cancer Clontech 9010320	9.5	13.7
Kidney NAT Clontech 9010321	47.0	5.1
Kidney Cancer Clontech 8120607	0.0	0.0
Kidney NAT Clontech 8120608	7.6	0.0
Normal Uterus GENPAK 061018	49.5	19.4
Uterus Cancer GENPAK 064011	14.7	0.0
Normal Thyroid Clontech A+ 6570-1 (7080817)	30.1	0.0

Thyroid Cancer GENPAK 064010	0.0	0.0
Thyroid Cancer INVITROGEN A302152	0.0	0.0
Thyroid NAT INVITROGEN A302153	0.0	5.7
Normal Breast GENPAK 061019	33.9	31.8
84877 Breast Cancer (OD04566)	37.6	15.3
Breast Cancer Res. Gen. 1024	100.0	100.0
85975 Breast Cancer (OD04590-01)	15.0	48.4
85976 Breast Cancer Mets (OD04590-03)	21.4	7.4
87070 Breast Cancer Metastasis (OD04655-05)	85.2	67.7
GENPAK Breast Cancer 064006	38.8	7.9
Breast Cancer Clontech 9100266	0.0	0.0
Breast NAT Clontech 9100265	0.0	0.0
Breast Cancer INVITROGEN A209073	9.8	0.0
Breast NAT INVITROGEN A2090734	59.8	13.8
97763 Breast cancer (OD06083)	52.2	34.0
97764 Breast cancer node metastasis (OD06083)	29.4	14.8
Normal Liver GENPAK 061009	27.5	10.7
Liver Cancer Research Genetics RNA 1026	0.0	0.0
Liver Cancer Research Genetics RNA 1025	27.3	15.8
Paired Liver Cancer Tissue Research Genetics RNA 6004-T	3.6	13.3
Paired Liver Tissue Research Genetics RNA 6004-N	3.8	0.0
Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0	12.6
Paired Liver Tissue Research Genetics RNA 6005-N	0.0	7.9
Liver Cancer GENPAK 064003	48.1	13.7
Normal Bladder GENPAK 061001	20.7	0.0
Bladder Cancer Research Genetics RNA 1023	0.0	0.0
Bladder Cancer INVITROGEN A302173	0.0	0.0
Normal Stomach GENPAK 061017	39.1	16.0
Gastric Cancer Clontech 9060397	0.0	0.0
NAT Stomach Clontech 9060396	20.0	0.0
Gastric Cancer Clontech 9060395	9.6	32.6
NAT Stomach Clontech 9060394	0.0	0.0
Gastric Cancer GENPAK 064005	30.9	16.0

Table 22F. Panel 2D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	2Dx4tm4851f_ag2201_a2		2Dx4tm4851f_ag2201_a2
Normal Colon GENPAK 061003	22.7	Kidney NAT Clontech 8120608	1.1
83219 CC Well to Mod Diff	7.6	Kidney Cancer Clontech	0.0

<u>(ODO3866)</u>		8120613	
<u>83220 CC NAT (ODO3866)</u>	1.4	Kidney NAT Clontech 8120614	4.8
<u>83221 CC Gr.2 rectosigmoid (ODO3868)</u>	1.0	Kidney Cancer Clontech 9010320	7.4
<u>83222 CC NAT (ODO3868)</u>	0.0	Kidney NAT Clontech 9010321	28.9
<u>83235 CC Mod Diff (ODO3920)</u>	1.9	Normal Uterus GENPAK 061018	0.0
<u>83236 CC NAT (ODO3920)</u>	6.9	Uterus Cancer GENPAK 064011	18.3
<u>83237 CC Gr.2 ascend colon (ODO3921)</u>	7.9	Normal Thyroid Clontech A+ 6570-1	3.0
<u>83238 CC NAT (ODO3921)</u>	2.0	Thyroid Cancer GENPAK 064010	0.0
<u>83241 CC from Partial Hepatectomy (ODO4309)</u>	0.9	Thyroid Cancer INVITROGEN A302152	1.5
<u>83242 Liver NAT (ODO4309)</u>	1.7	Thyroid NAT INVITROGEN A302153	7.1
<u>87472 Colon mets to lung (OD04451-01)</u>	4.3	Normal Breast GENPAK 061019	20.4
<u>87473 Lung NAT (OD04451-02)</u>	3.5	84877 Breast Cancer (OD04566)	10.3
<u>Normal Prostate Clontech A+ 6546-1</u>	5.0	85975 Breast Cancer (OD04590-01)	14.4
<u>84140 Prostate Cancer (OD04410)</u>	15.5	85976 Breast Cancer Mets (OD04590-03)	34.4
<u>84141 Prostate NAT (OD04410)</u>	8.8	87070 Breast Cancer Metastasis (OD04655-05)	69.8
<u>87073 Prostate Cancer (OD04720-01)</u>	12.0	GENPAK Breast Cancer 064006	30.8
<u>87074 Prostate NAT (OD04720-02)</u>	17.2	Breast Cancer Res. Gen. 1024	100.0
<u>Normal Lung GENPAK 061010</u>	6.9	Breast Cancer Clontech 9100266	11.7
<u>83239 Lung Met to Muscle (ODO4286)</u>	4.2	Breast NAT Clontech 9100265	6.8
<u>83240 Muscle NAT (ODO4286)</u>	4.4	Breast Cancer INVITROGEN A209073	12.2
<u>84136 Lung Malignant Cancer (OD03126)</u>	3.2	Breast NAT INVITROGEN A2090734	8.9
<u>84137 Lung NAT (OD03126)</u>	10.5	Normal Liver GENPAK 061009	10.4
<u>84871 Lung Cancer (OD04404)</u>	1.0	Liver Cancer GENPAK 064003	5.6
<u>84872 Lung NAT (OD04404)</u>	10.4	Liver Cancer Research Genetics RNA 1025	2.6
<u>84875 Lung Cancer (OD04565)</u>	3.8	Liver Cancer Research Genetics RNA 1026	2.1
<u>84876 Lung NAT (OD04565)</u>	16.5	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	3.0

85950 Lung Cancer (OD04237-01)	3.0	Paired Liver Tissue Research Genetics RNA 6004-N	3.9
85970 Lung NAT (OD04237-02)	4.3	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0
83255 Ocular Mel Met to Liver (ODO4310)	4.5	Paired Liver Tissue Research Genetics RNA 6005-N	0.7
83256 Liver NAT (ODO4310)	2.0	Normal Bladder GENPAK 061001	2.7
84139 Melanoma Mets to Lung (OD04321)	0.0	Bladder Cancer Research Genetics RNA 1023	6.2
84138 Lung NAT (OD04321)	2.6	Bladder Cancer INVITROGEN A302173	13.8
Normal Kidney GENPAK 061008	20.3	87071 Bladder Cancer (OD04718-01)	1.8
83786 Kidney Ca, Nuclear grade 2 (OD04338)	1.0	87072 Bladder Normal Adjacent (OD04718-03)	5.0
83787 Kidney NAT (OD04338)	2.8	Normal Ovary Res. Gen.	0.7
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	9.2	Ovarian Cancer GENPAK 064008	3.8
83789 Kidney NAT (OD04339)	18.8	87492 Ovary Cancer (OD04768-07)	6.7
83790 Kidney Ca, Clear cell type (OD04340)	15.3	87493 Ovary NAT (OD04768-08)	0.0
83791 Kidney NAT (OD04340)	22.7	Normal Stomach GENPAK 061017	14.4
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Gastric Cancer Clontech 9060358	0.0
83793 Kidney NAT (OD04348)	5.2	NAT Stomach Clontech 9060359	2.1
87474 Kidney Cancer (OD04622-01)	1.9	Gastric Cancer Clontech 9060395	1.4
87475 Kidney NAT (OD04622-03)	0.0	NAT Stomach Clontech 9060394	6.0
85973 Kidney Cancer (OD04450-01)	10.3	Gastric Cancer Clontech 9060397	5.6
85974 Kidney NAT (OD04450-03)	13.8	NAT Stomach Clontech 9060396	4.4
Kidney Cancer Clontech 8120607	0.7	Gastric Cancer GENPAK 064005	27.8

Table 22G. Panel 4D

Tissue Name	Relative Expression(%) 4dtm4960t_ag2433	Relative Expression(%) 4dx4tm4508f_ag2201_a2	Relative Expression(%) 4dx4tm5031f_ag2479_b1	Relative Expression(%) 4dx4tm5003t_ag2537_a1
93768_Secondary Th1_anti-CD28/anti-CD3	2.1	2.6	7.5	9.5

93769_Secondary Th2_anti-CD28/anti-CD3	16.7	55.2	29.2	16.3
93770_Secondary Tr1_anti-CD28/anti-CD3	21.8	25.0	39.3	53.6
93573_Secondary Th1_resting day 4-6 in IL-2	1.8	5.7	20.2	10.1
93572_Secondary Th2_resting day 4-6 in IL-2	19.5	13.4	8.0	24.9
93571_Secondary Tr1_resting day 4-6 in IL-2	7.8	24.8	33.1	12.7
93568_primary Th1_anti-CD28/anti-CD3	9.7	18.8	10.3	3.0
93569_primary Th2_anti-CD28/anti-CD3	18.3	24.6	20.2	17.4
93570_primary Tr1_anti-CD28/anti-CD3	18.4	27.9	8.6	14.2
93565_primary Th1_resting dy 4-6 in IL-2	100.0	100.0	35.3	100.0
93566_primary Th2_resting dy 4-6 in IL-2	29.7	72.1	40.5	45.0
93567_primary Tr1_resting dy 4-6 in IL-2	0.0	17.3	21.3	17.7
93351_CD45RA CD4 lymphocyte_anti-CD28/anti-CD3	4.1	14.6	16.3	16.0
93352_CD45RO CD4 lymphocyte_anti-CD28/anti-CD3	17.6	26.4	33.4	6.3
93251_CD8 Lymphocytes_anti-CD28/anti-CD3	24.5	23.9	40.9	14.2
93353_chronic CD8 Lymphocytes 2ry_resting dy 4-6 in IL-2	51.8	25.6	42.8	26.7
93574_chronic CD8 Lymphocytes 2ry_activated CD3/CD28	4.8	10.2	5.5	6.7
93354_CD4_none	5.2	8.8	12.4	5.2
93252_Secondary Th1/Th2/Tr1_anti-CD95 CH11	12.2	32.0	9.3	5.8
93103_LAK cells_resting	2.6	5.0	10.6	3.9
93788_LAK cells_IL-2	33.2	59.9	71.8	39.9
93787_LAK cells_IL-2+IL-12	32.8	31.1	38.0	53.9
93789_LAK cells_IL-2+IFN gamma	37.4	11.3	36.2	56.7
93790_LAK cells_IL-2+ IL-18	29.1	23.4	40.9	22.8
93104_LAK cells_PMA/ionomycin and IL-18	2.5	0.0	7.2	5.1
93578_NK Cells IL-2_resting	21.5	17.3	39.7	19.5

93109_Mixed Lymphocyte Reaction Two Way MLR	62.0	92.1	100.0	62.4
93110_Mixed Lymphocyte Reaction Two Way MLR	20.3	32.8	26.5	30.8
93111_Mixed Lymphocyte Reaction Two Way MLR	8.6	18.5	17.6	16.3
93112_Mononuclear Cells (PBMCs) resting	2.4	8.4	0.0	7.6
93113_Mononuclear Cells (PBMCs) PWM	26.1	37.4	70.4	45.9
93114_Mononuclear Cells (PBMCs) PHA-L	8.6	6.0	24.9	14.6
93249_Ramos (B cell) none	11.0	25.1	20.5	7.6
93250_Ramos (B cell) ionomycin	35.4	41.9	44.8	45.8
93349_B lymphocytes PWM	2.0	17.6	8.3	20.3
93350_B lymphocytes_CD40L and IL-4	17.3	21.4	16.9	25.4
92665_EOL-1 (Eosinophil) dbcAMP differentiated	0.0	2.7	0.0	0.0
93248_EOL-1 (Eosinophil) dbcAMP/PMAion omycin	7.0	4.7	0.0	0.0
93356_Dendritic Cells none	1.8	13.4	16.7	14.7
93355_Dendritic Cells_LPS 100 ng/ml	14.2	10.7	2.4	11.6
93775_Dendritic Cells_anti- CD40	12.2	12.4	10.4	3.0
93774_Monocytes_resting	0.0	2.5	7.8	4.3
93776_Monocytes_LPS 50 ng/ml	18.8	12.7	25.1	10.1
93581_Macrophages resting	7.9	8.3	11.0	10.9
93582_Macrophages_LPS 100 ng/ml	3.8	3.2	6.4	0.0
93098_HUVEC (Endothelial) none	2.3	3.1	0.0	3.7
93099_HUVEC (Endothelial) starved	4.6	8.6	11.5	6.9
93100_HUVEC (Endothelial) IL-1b	5.2	2.4	5.5	0.6
93779_HUVEC (Endothelial) IFN gamma	13.6	15.8	33.0	21.2
93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	0.0	0.0	4.7	0.0
93101_HUVEC (Endothelial)_TNF alpha + IL4	0.0	11.4	8.4	0.0
93781_HUVEC	4.8	0.0	19.5	5.4

(Endothelial)_IL-11				
93583_Lung Microvascular Endothelial Cells none	14.4	29.9	25.7	12.8
93584_Lung Microvascular Endothelial Cells TNFa (4 ng/ml) and IL1b (1 ng/ml)	25.7	26.1	18.4	26.0
92662_Microvascular Dermal endothelium none	2.0	3.3	3.2	3.9
92663_Microvascular Dermal endothelium TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	6.5	10.6	9.0
93773_Bronchial epithelium TNFa (4 ng/ml) and IL1b (1 ng/ml) **	16.3	16.1	11.8	15.8
93347_Small Airway Epithelium none	5.7	8.2	9.2	0.0
93348_Small Airway Epithelium TNFa (4 ng/ml) and IL1b (1 ng/ml)	23.8	31.1	39.1	32.7
92668_Coronary Artery SMC resting	12.6	0.0	3.2	1.3
92669_Coronary Artery SMC TNFa (4 ng/ml) and IL1b (1 ng/ml)	6.4	0.0	0.0	6.1
93107_astrocytes resting	0.0	2.7	5.7	2.9
93108_astrocytes TNFa (4 ng/ml) and IL1b (1 ng/ml)	10.7	3.3	0.0	8.1
92666_KU-812 (Basophil) resting	6.6	0.0	3.3	3.4
92667_KU-812 (Basophil) PMA/ionoycin	5.5	0.0	8.3	3.3
93579_CCD1106 (Keratinocytes) none	7.2	6.7	3.4	7.0
93580_CCD1106 (Keratinocytes) TNFa and IFNg **	20.9	0.0	3.6	0.0
93791_Liver Cirrhosis	22.5	26.2	39.5	15.4
93792_Lupus Kidney	2.5	1.6	9.9	3.4
93577_NCI-H292	6.7	10.8	10.0	12.0
93358_NCI-H292_IL-4	17.4	16.6	13.3	16.8
93360_NCI-H292_IL-9	5.6	5.4	5.0	16.7
93359_NCI-H292_IL-13	0.0	6.6	0.0	6.8
93357_NCI-H292_IFN gamma	5.4	2.9	6.8	0.0
93777_HPAEC -	0.0	6.4	4.6	2.7
93778_HPAEC_IL-1 beta/TNA alpha	9.9	8.5	19.7	3.1
93254_Normal Human Lung Fibroblast none	5.5	0.0	2.0	9.0

93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0	11.8	0.0	0.0
93257_Normal Human Lung Fibroblast_IL-4	8.7	16.8	22.8	11.2
93256_Normal Human Lung Fibroblast_IL-9	8.2	9.4	3.3	6.2
93255_Normal Human Lung Fibroblast_IL-13	13.2	5.0	1.5	2.5
93258_Normal Human Lung Fibroblast_IFN gamma	7.1	19.8	19.3	19.2
93106_Dermal Fibroblasts CCD1070_resting	26.1	26.6	35.2	13.9
93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	19.3	40.3	31.2	21.0
93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	10.0	13.0	18.1	15.7
93772_dermal fibroblast_IFN gamma	9.8	19.3	11.2	21.3
93771_dermal fibroblast_IL-4	2.0	25.6	36.1	28.6
93260_IBD Colitis 2	6.3	10.9	2.4	0.0
93261_IBD Crohns	0.0	0.0	7.5	2.5
735010_Colon_normal	15.8	6.5	20.1	12.9
735019_Lung_none	16.5	32.5	24.9	23.9
64028-1_Thymus_none	5.6	16.1	28.6	20.7
64030-1_Kidney_none	18.2	13.7	21.8	10.5

Table 22I. Panel CNS_neurodegeneration_v1.0

Tissue Name	Relative Expression(%)	Relative Expression(%)	Relative Expression(%)
	tm7012t_ag2537_b2	tm7041f_ag2479_a1	tm7000f_ag2201_b2_s2
AD 1 Hippo	1.6	1.8	0.0
AD 2 Hippo	4.0	8.0	8.3
AD 3 Hippo	6.3	3.3	4.1
AD 4 Hippo	6.1	4.5	2.6
AD 5 hippo	21.7	22.3	16.5
AD 6 Hippo	25.9	58.0	46.7
Control 2 Hippo	4.3	1.5	2.3
Control 4 Hippo	7.1	5.9	16.9
Control (Path) 3 Hippo	6.4	1.7	1.9
AD 1 Temporal Ctx	4.4	3.6	2.5
AD 2 Temporal Ctx	2.2	5.1	2.9
AD 3 Temporal Ctx	6.6	3.4	2.2
AD 4 Temporal Ctx	16.9	18.1	18.1

AD 5 Inf Temporal Ctx	14.0	29.3	15.6
AD 5 SupTemporal Ctx	2.2	12.7	17.4
AD 6 Inf Temporal Ctx	62.4	69.4	40.8
AD 6 Sup Temporal Ctx	100.0	100.0	100.0
Control 1 Temporal Ctx	0.0	0.0	2.1
Control 2 Temporal Ctx	10.8	7.1	2.3
Control 3 Temporal Ctx	5.4	10.5	3.4
Control 4 Temporal Ctx	6.9	7.5	10.9
Control (Path) 1 Temporal Ctx	8.5	14.6	14.6
Control (Path) 2 Temporal Ctx	5.5	12.9	15.3
Control (Path) 3 Temporal Ctx	0.0	0.0	5.0
Control (Path) 4 Temporal Ctx	18.4	18.6	13.8
AD 1 Occipital Ctx	0.0	5.0	2.7
AD 2 Occipital Ctx (Missing)	0.0	0.0	0.0
AD 3 Occipital Ctx	4.8	2.0	13.6
AD 4 Occipital Ctx	17.1	19.2	12.8
AD 5 Occipital Ctx	11.7	22.3	21.9
AD 6 Occipital Ctx	3.6	2.1	6.1
Control 1 Occipital Ctx	0.4	3.5	0.0
Control 2 Occipital Ctx	2.1	12.5	1.9
Control 3 Occipital Ctx	4.0	7.7	2.0
Control 4 Occipital Ctx	8.9	6.2	9.1
Control (Path) 1 Occipital Ctx	14.6	15.0	9.4
Control (Path) 2 Occipital Ctx	10.0	14.9	18.2
Control (Path) 3 Occipital Ctx	0.0	1.8	0.0
Control (Path) 4 Occipital Ctx	7.6	15.9	19.0
Control 1 Parietal Ctx	1.3	1.8	0.0
Control 2 Parietal Ctx	10.6	8.3	15.9
Control 3 Parietal Ctx	3.5	8.5	5.5
Control (Path) 1 Parietal Ctx	5.6	11.1	5.5
Control (Path) 2 Parietal Ctx	12.4	8.6	5.6
Control (Path) 3 Parietal Ctx	1.5	2.8	1.6
Control (Path) 4 Parietal Ctx	15.4	25.2	20.6

Panel 1.3D Summary Ag2479/Ag2537/Ag2201 Results from three experiments using different probe/primer sets show somewhat disparate results, most likely because the levels of gene expression are very low in this panel. Using Ag2201 and Ag2537, expression of the CG50193-01 gene is highest in a brain cancer cell line (CT=34). In addition, there is low but significant expression in an additional sample derived from a brain cancer cell line. Other apparent expression is below the level of reliable evaluation. Of note is the observation that

both of the cell lines showing substantial CG50193-01 gene expression are derived from a type of brain cancer called glioblastoma. Thus, the expression of this gene could be used to distinguish between glioblastoma derived samples and other samples. Moreover, therapeutic modulation of the expression or function of the CG50193-01 gene or its protein product, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of glioblastoma. Using Ag2479, expression is highest in spleen (CT=34), with low but significant expression also seen in a melanoma cell line as well as a brain cancer cell line. Other apparent expression is below the level of reliable evaluation. Thus, therapeutic modulation of the expression or function of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of use in the treatment of brain cancer or melanoma. Ag2433 Expression of this gene in panel 1.3D is low/undetectable (Ct values >35) in all samples (data not shown).

Panel 2.2 Summary Ag2479/Ag2433 Two experiments with two different probe and primer sets produce results that are in excellent agreement. In both runs, expression is limited to samples derived from breast cancer. Thus, expression of the CG50193-01 gene could be used to distinguish sample derived from breast cancer from other samples. Moreover, therapeutic modulation of the expression or function of this gene, through the use of small molecule drugs or antibodies, might be of use in the treatment of breast cancer.

Panel 2D Summary Ag2201 Expression of the CG50193-01 gene is highest in a sample derived from a breast cancer. In addition, a number of other breast cancer samples show substantial expression, including samples of cancer tissue with matched samples derived from normal adjacent tissue. In all these samples, the CG50193-01 gene appears to be over-expressed in the cancerous tissue. This result agrees with the expression profile detected in Panel 2.2 and suggests that expression of this gene could be used to distinguish breast cancer tissue from other tissues, perhaps as a diagnostic marker for the presence of breast cancer. Moreover, therapeutic inhibition of the protein encoded by the CG50193-01 gene may be effective in the treatment of breast cancer.

Panel 4D Summary Ag2479/Ag2537/Ag2201/Ag2433 The CG50193-01 gene is expressed in Panel 4D at moderate to low levels in numerous independent preparations of

activated B cells, resting and activated T cells, and activated lymphokine-activated killer cells. This pattern of restricted expression suggests that specific antibodies and small molecule drugs that inhibit the function of the protein encoded by the CG50193-01 gene may be useful in reducing or eliminating inflammation and autoimmune disease symptoms in patients with Crohn's disease, inflammatory bowel disease, asthma, psoriasis, and rheumatoid arthritis.

Panel CNS_neurodegeneration_v1.0 Summary Ag2479/Ag2537/Ag2201 The CG50193-01 gene is expressed primarily in the cerebellum and also shows increased expression in the hippocampus and inferior temporal cortex of some brains affected with Alzheimer's disease when compared to normal baseline expression in unaffected brains. The hippocampus is an important anatomical focus of Alzheimer's pathology, indicating that the CG50193-01 gene product may be an important biochemical component of the disease. GPCRs are readily targetable with drugs, and regulate many specific brain processes, including signaling processes that are currently the target of FDA-approved pharmaceuticals that treat Alzheimer's disease, such as the cholinergic system. The major mechanisms proposed for Abeta-induced cytotoxicity involve the loss of Ca²⁺ homeostasis and the generation of reactive oxygen species (ROS). The changes in Ca²⁺ homeostasis could be the result of changes in G-protein-driven releases of second messengers. Thus, targeting this class of molecule can have therapeutic potential in Alzheimer's disease treatment. In particular, the increased expression of the CG50193-01 gene in some brains affected by Alzheimer's indicates potential therapeutic value to drugs that target this GPCR. Normal expression of this gene in the cerebellum suggests that this GPCR may also be effectively targeted to treat diseases involving the cerebellum, including spinocerebellar ataxias, batten disease, and Niemann-Pick disease.

GPCR9 (also referred to as CG50203-01)

Expression of gene CG50203-01 was assessed using the primer-probe sets Ag2484, Ag1783, Ag1781, and Ag1583 described in Tables 23A and 23B. Results from RTQ-PCR runs are shown in Tables 23C and 23D.

Table 23A Probe Name Ag2484 (SEQ ID NO: 186, 187, 188)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GGCTACTTGTACAATGGAATGG-3'	58.5	22	475
Probe	FAM-5'-CAAGCCACAGAACCAACGATAATGCA-3'-TAMRA	69.7	26	507
Reverse	5'-TCAACCATCATGAACCCCTAGAG-3'	59	22	540

Table 23B Probe Name Ag1783/1781/1583 (identical sequences) (SEQ ID NO: 189, 190, 191)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GATGCTCAACTTCTGGTCTTTG-3'	59	22	69
Probe	TET-5'-CATCCTCCCTGGAAATTCTCATCA-3'-TAMRA	69	26	116
Reverse	5'-CAGGGTCTGACTTTATGGTGAA-3'	59.1	22	144

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Table 23C. Panel 1.3D

Tissue Name	Relative Expression(%)	Relative Expression(%)
	1.3dx4tm5599 t_ag1781_a1	1.3dx4tm5599 t_ag1783_a2
Liver adenocarcinoma	2.0	0.0
Pancreas	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0
Adrenal gland	0.0	0.0
Thyroid	0.0	0.0
Salivary gland	0.0	0.0
Pituitary gland	0.0	0.0
Brain (fetal)	0.0	0.0
Brain (whole)	0.0	0.0
Brain (amygdala)	0.0	0.0
Brain (cerebellum)	0.0	0.0
Brain (hippocampus)	0.0	0.0
Brain (substantia nigra)	0.0	0.0
Brain (thalamus)	0.0	0.0
Cerebral Cortex	0.0	0.0
Spinal cord	0.0	0.0
CNS ca. (glio/astro) U87-MG	0.0	0.0
CNS ca. (glio/astro) U-118-MG	0.0	0.0
CNS ca. (astro) SW1783	0.0	0.0
CNS ca.* (neuro; met) SK-N-AS	0.0	0.0
CNS ca. (astro) SF-539	0.0	0.0
CNS ca. (astro) SNB-75	0.0	0.0
CNS ca. (glio) SNB-19	0.0	0.0

CNS ca. (glio) U251	0.0	14.5
CNS ca. (glio) SF-295	0.0	0.0
Heart (fetal)	0.0	0.0
Heart	0.0	0.0
Fetal Skeletal	0.0	0.0
Skeletal muscle	0.0	0.0
Bone marrow	0.0	0.0
Thymus	0.0	0.0
Spleen	100.0	100.0
Lymph node	0.0	0.0
Colorectal	2.0	0.0
Stomach	0.0	0.0
Small intestine	0.0	0.0
Colon ca. SW480	0.0	0.0
Colon ca.* (SW480 met)SW620	0.0	0.0
Colon ca. HT29	0.0	0.0
Colon ca. HCT-116	0.0	0.0
Colon ca. CaCo-2	0.0	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	0.0
Colon ca. HCC-2998	0.0	0.0
Gastric ca.* (liver met) NCI-N87	0.0	11.4
Bladder	0.0	0.0
Trachea	0.0	0.0
Kidney	0.0	0.0
Kidney (fetal)	0.0	0.0
Renal ca. 786-0	0.0	0.0
Renal ca. A498	0.0	0.0
Renal ca. RXF 393	7.6	0.0
Renal ca. ACHN	0.0	0.0
Renal ca. UO-31	0.0	0.0
Renal ca. TK-10	0.0	0.0
Liver	0.0	0.0
Liver (fetal)	0.0	0.0
Liver ca. (hepatoblast) HepG2	0.0	0.0
Lung	0.0	0.0
Lung (fetal)	0.0	0.0
Lung ca. (small cell) LX-1	0.0	0.0
Lung ca. (small cell) NCI-H69	0.0	0.0
Lung ca. (s.cell var.) SHP-77	0.0	0.0
Lung ca. (large cell)NCI-H460	0.0	0.0
Lung ca. (non-sm. cell) A549	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	0.0	0.0

Lung ca (non-s.cell) HOP-62	0.0	0.0
Lung ca. (non-s.cl) NCI-H522	0.0	0.0
Lung ca. (squam.) SW 900	0.0	0.0
Lung ca. (squam.) NCI-H596	0.0	0.0
Mammary gland	0.0	0.0
Breast ca.* (pl. effusion) MCF-7	0.0	0.0
Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0
Breast ca.* (pl. effusion) T47D	0.0	0.0
Breast ca. BT-549	0.0	0.0
Breast ca. MDA-N	0.0	0.0
Ovary	0.0	0.0
Ovarian ca. OVCAR-3	0.0	0.0
Ovarian ca. OVCAR-4	0.0	0.0
Ovarian ca. OVCAR-5	0.0	0.0
Ovarian ca. OVCAR-8	0.0	0.0
Ovarian ca. IGROV-1	0.0	0.0
Ovarian ca.* (ascites) SK-OV-3	5.5	32.3
Uterus	0.0	0.0
Placenta	0.0	0.0
Prostate	0.0	0.0
Prostate ca.* (bone met)PC-3	0.0	0.0
Testis	1.1	13.3
Melanoma Hs688(A).T	0.0	0.0
Melanoma* (met) Hs688(B).T	0.0	0.0
Melanoma UACC-62	0.0	0.0
Melanoma M14	0.0	0.0
Melanoma LOX IMVI	0.0	0.0
Melanoma* (met) SK-MEL-5	0.0	0.0
Adipose	0.0	0.0

Table 23D. Panel 4D

Tissue Name	Relative Expression(%)	Relative Expression(%)
	4dx4tm5547_ ag1781_b1	4dx4tm_ ag1783_b2
93768_Secondary Th1_anti-CD28/anti-CD3	0.0	0.0
93769_Secondary Th2_anti-CD28/anti-CD3	0.0	0.0
93770_Secondary Tr1_anti-CD28/anti-CD3	0.0	13.3
93573_Secondary Th1_resting day 4-6 in IL-2	0.0	0.0
93572_Secondary Th2_resting day 4-6 in IL-2	0.0	0.0
93571_Secondary Tr1_resting day 4-6 in IL-2	0.0	0.0
93568_primary Th1_anti-CD28/anti-CD3	0.0	0.0

93569	primary Th2 anti-CD28/anti-CD3	0.0	0.0
93570	primary Tr1 anti-CD28/anti-CD3	0.0	0.0
93565	primary Th1 resting dy 4-6 in IL-2	0.0	0.0
93566	primary Th2 resting dy 4-6 in IL-2	0.0	0.0
93567	primary Tr1 resting dy 4-6 in IL-2	0.0	0.0
93351	CD45RA CD4 lymphocyte anti-CD28/anti-CD3	0.0	9.4
93352	CD45RO CD4 lymphocyte anti-CD28/anti-CD3	0.0	0.0
93251	CD8 Lymphocytes anti-CD28/anti-CD3	0.0	0.0
93353	chronic CD8 Lymphocytes 2ry resting dy 4-6 in IL-2	0.0	0.0
93574	chronic CD8 Lymphocytes 2ry activated CD3/CD28	0.0	0.0
93354	CD4 none	0.0	0.0
93252	Secondary Th1/Th2/Tr1 anti-CD95 CH11	0.0	0.0
93103	LAK cells resting	0.0	0.0
93788	LAK cells IL-2	0.0	0.0
93787	LAK cells IL-2+IL-12	0.0	0.0
93789	LAK cells IL-2+IFN gamma	0.0	0.0
93790	LAK cells IL-2+ IL-18	0.0	0.0
93104	LAK cells PMA/ionomycin and IL-18	0.0	0.0
93578	NK Cells IL-2 resting	4.8	0.0
93109	Mixed Lymphocyte Reaction Two Way MLR	0.0	6.5
93110	Mixed Lymphocyte Reaction Two Way MLR	0.0	0.0
93111	Mixed Lymphocyte Reaction Two Way MLR	0.0	0.0
93112	Mononuclear Cells (PBMCs) resting	0.0	0.0
93113	Mononuclear Cells (PBMCs) PWM	0.0	0.0
93114	Mononuclear Cells (PBMCs) PHA-L	0.0	0.0
93249	Ramos (B cell) none	0.0	0.0
93250	Ramos (B cell) ionomycin	0.0	0.0
93349	B lymphocytes PWM	0.0	0.0
93350	B lymphocytes CD40L and IL-4	0.0	8.2
92665	EOL-1 (Eosinophil) dbcAMP differentiated	0.0	0.0
93248	EOL-1 (Eosinophil) dbcAMP/PMAionomycin	0.0	0.0
93356	Dendritic Cells none	0.0	0.0
93355	Dendritic Cells LPS 100 ng/ml	0.0	0.0
93775	Dendritic Cells anti-CD40	0.0	0.0
93774	Monocytes resting	0.0	0.0
93776	Monocytes LPS 50 ng/ml	0.0	0.0
93581	Macrophages resting	0.0	0.0
93582	Macrophages LPS 100 ng/ml	0.0	0.0
93098	HUVEC (Endothelial) none	0.0	0.0
93099	HUVEC (Endothelial) starved	0.0	0.0
93100	HUVEC (Endothelial) IL-1b	0.0	0.0
93779	HUVEC (Endothelial) IFN gamma	0.0	0.0

93102 HUVEC (Endothelial) TNF alpha + IFN gamma	0.0	0.0
93101 HUVEC (Endothelial) TNF alpha + IL4	0.0	0.0
93781 HUVEC (Endothelial) IL-11	0.0	0.0
93583 Lung Microvascular Endothelial Cells none	0.0	0.0
93584 Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
92662 Microvascular Dermal endothelium none	0.0	0.0
92663_Microsvascular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93773 Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml) **	0.0	0.0
93347 Small Airway Epithelium none	0.0	0.0
93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	2.9	0.0
92668 Coronary Artery SMC_resting	0.0	0.0
92669_Coronary Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
93107 astrocytes resting	0.0	0.0
93108 astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	0.0	0.0
92666_KU-812 (Basophil) resting	0.0	0.0
92667_KU-812 (Basophil) PMA/ionoycin	0.0	0.0
93579 CCD1106 (Keratinocytes) none	0.0	0.0
93580 CCD1106 (Keratinocytes) TNFa and IFNg **	0.0	0.0
93791 Liver Cirrhosis	100.0	100.0
93792 Lupus Kidney	0.0	0.0
93577 NCI-H292	0.0	0.0
93358 NCI-H292 IL-4	0.0	0.0
93360 NCI-H292 IL-9	0.0	0.0
93359 NCI-H292 IL-13	0.0	0.0
93357 NCI-H292 IFN gamma	0.0	0.0
93777 HPAEC -	0.0	0.0
93778 HPAEC IL-1 beta/TNA alpha	0.0	0.0
93254 Normal Human Lung Fibroblast none	0.0	0.0
93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	0.0	0.0
93257 Normal Human Lung Fibroblast IL-4	0.0	0.0
93256 Normal Human Lung Fibroblast IL-9	0.0	0.0
93255 Normal Human Lung Fibroblast IL-13	0.0	0.0
93258 Normal Human Lung Fibroblast IFN gamma	0.0	0.0
93106 Dermal Fibroblasts CCD1070 resting	0.0	0.0
93361 Dermal Fibroblasts CCD1070 TNF alpha 4 ng/ml	9.5	0.0
93105 Dermal Fibroblasts CCD1070 IL-1 beta 1 ng/ml	0.0	0.0
93772 dermal fibroblast IFN gamma	0.0	0.0
93771 dermal fibroblast IL-4	0.0	0.0

93260 IBD Colitis 2	19.3	38.7
93261 IBD Crohns	3.1	18.4
735010 Colon normal	11.9	7.4
735019 Lung none	0.0	0.0
64028-1 Thymus none	4.8	0.0
64030-1 Kidney none	0.0	0.0

Panel 1.3D Summary Ag1783/Ag1781 Two experiments with the same probe and primer set are in good agreement, with significant expression of the CG50203-01 gene is limited to the spleen. This suggests that expression of the gene could be used to differentiate spleen tissue from other tissue types. **Ag2484/Ag1583** Expression of the CG50203-01 gene in panel 1.3D is low/undetectable (CT values >35) in all samples (data not shown).

Panel 2.2 Summary Ag1783/Ag1781/Ag1583 Expression of the CG50203-01 gene in panel 2.2 is low/undetectable (CT values >35) in all samples (data not shown).

Panel 4D Summary Ag1783/Ag1781 Two experiments using the same probe and primer set are in very good agreement, with significant expression of the CG50203-01 gene limited to a sample from liver cirrhosis. This result suggests that the protein encoded by the CG50203-01 gene may play a role in liver cirrhosis. Thus, small molecule drugs or specific antibodies to the CG50203-01 gene product may be effective in the treatment of liver cirrhosis. **Ag1583/Ag2484** Expression of this gene in panel 4D is low/undetectable (CT values >35) in all samples (data not shown).

Panel CNS_neurodegeneration_v1.0 Summary Ag2484 Expression of the CG50203-01 gene in panel CNS_neurodegeneration is low/undetectable (CT values >35) in all samples (data not shown).

GPCR10 (also referred to as CG50197-01 and CG50197-02)

Expression of gene CG50197-01 and variant CG50197-02 was assessed using the primer-probe sets Ag2482 and Ag2347, described in Tables 24A, and 24B. Results from RTQ-PCR runs are shown in Tables 24C, 24D, 24E, and 24F.

Table 24A Probe Name Ag2482 (SEQ ID NO: 192, 193, 194)

Primers	Sequences	TM	Length	Start Position
Forward	5' - GATGCCAATCTCTCTGGTATCA - 3'	59.2	22	720
Probe	FAM - 5' - AACCCAGAAAATGCCAGCACAGTG - 3' - TAMRA	68.4	24	742
Reverse	5' - CTGTCACAGACTTAGGCCCTTG - 3'	59	22	781

Table 24B Probe Name Ag2347 (SEQ ID NO: 195, 196, 197)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GTCTCCATGGCTGGATCTCTAT-3'	59.6	22	73
Probe	FAM-5'-TCCCTTCTGCTTCATCTACCTGACAG-3'-TAMRA	66.1	26	95
Reverse	5'-TACAAATGACGTGGAGAATGGT-3'	59.4	22	138

Table 24C. Panel 1.3D

Tissue Name	Relative Expression(%)	
	1.3dx4tm4877 f_ag2482_a2	1.3dx4tm545 7f_ag2482_b2
Liver adenocarcinoma	0.0	0.0
Pancreas	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0
Adrenal gland	0.0	0.0
Thyroid	0.0	0.0
Salivary gland	0.0	0.0
Pituitary gland	0.0	0.0
Brain (fetal)	0.0	0.0
Brain (whole)	0.0	0.0
Brain (amygdala)	0.0	0.0
Brain (cerebellum)	0.0	0.0
Brain (hippocampus)	0.0	0.0
Brain (substantia nigra)	0.0	0.0
Brain (thalamus)	0.0	0.0
Cerebral Cortex	0.0	0.0
Spinal cord	0.0	0.0
CNS ca. (glio/astro) U87-MG	0.0	0.0
CNS ca. (glio/astro) U-118-MG	0.0	0.0
CNS ca. (astro) SW1783	0.0	0.0
CNS ca.* (neuro; met) SK-N-AS	0.0	2.4
CNS ca. (astro) SF-539	0.0	3.9
CNS ca. (astro) SNB-75	0.0	0.0
CNS ca. (glio) SNB-19	0.0	0.0
CNS ca. (glio) U251	0.0	0.0
CNS ca. (glio) SF-295	0.0	0.0
Heart (fetal)	0.0	0.0
Heart	0.0	0.0
Fetal Skeletal	0.0	0.0
Skeletal muscle	0.0	0.0
Bone marrow	0.0	0.0

Thymus	0.0	0.0
Spleen	0.0	0.0
Lymph node	0.0	0.0
Colorectal	2.6	0.0
Stomach	0.0	0.0
Small intestine	0.0	0.0
Colon ca. SW480	1.9	0.0
Colon ca.* (SW480 met)SW620	0.0	0.0
Colon ca. HT29	0.0	0.0
Colon ca. HCT-116	0.0	0.0
Colon ca. CaCo-2	0.0	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	4.1
Colon ca. HCC-2998	0.0	0.0
Gastric ca.* (liver met) NCI-N87	0.0	0.0
Bladder	4.9	0.0
Trachea	2.0	0.0
Kidney	0.0	0.0
Kidney (fetal)	2.4	0.0
Renal ca. 786-0	0.0	0.0
Renal ca. A498	0.0	0.0
Renal ca. RXF 393	0.0	0.0
Renal ca. ACHN	0.0	0.0
Renal ca. UO-31	6.4	0.0
Renal ca. TK-10	0.0	0.0
Liver	0.0	0.0
Liver (fetal)	0.0	0.0
Liver ca. (hepatoblast) HepG2	0.0	0.0
Lung	0.0	0.0
Lung (fetal)	0.0	0.0
Lung ca. (small cell) LX-1	0.0	0.0
Lung ca. (small cell) NCI-H69	16.1	2.2
Lung ca. (s.cell var.) SHP-77	100.0	100.0
Lung ca. (large cell)NCI-H460	0.0	0.0
Lung ca. (non-sm. cell) A549	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	0.0	0.0
Lung ca (non-s.cell) HOP-62	0.0	0.0
Lung ca. (non-s.cl) NCI-H522	0.0	0.0
Lung ca. (squam.) SW 900	0.0	0.0
Lung ca. (squam.) NCI-H596	0.0	11.5
Mammary gland	0.0	0.0
Breast ca.* (pl. effusion) MCF-7	0.0	0.0
Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0

Breast ca.* (pl. effusion) T47D	0.0	0.0
Breast ca. BT-549	0.0	3.7
Breast ca. MDA-N	0.0	0.0
Ovary	0.0	0.0
Ovarian ca. OVCAR-3	0.0	0.0
Ovarian ca. OVCAR-4	0.0	0.0
Ovarian ca. OVCAR-5	0.0	0.0
Ovarian ca. OVCAR-8	0.0	0.0
Ovarian ca. IGROV-1	0.0	0.0
Ovarian ca.* (ascites) SK-OV-3	0.0	0.0
Uterus	0.0	0.0
Placenta	0.0	0.0
Prostate	5.7	0.0
Prostate ca.* (bone met)PC-3	0.0	0.0
Testis	2.3	3.4
Melanoma Hs688(A).T	0.0	0.0
Melanoma* (met) Hs688(B).T	0.0	0.0
Melanoma UACC-62	0.0	0.0
Melanoma M14	0.0	0.0
Melanoma LOX IMVI	0.0	0.0
Melanoma* (met) SK-MEL-5	0.0	0.0
Adipose	0.0	0.0

Table 24D. Panel 2.2

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	2.2x4tm6421f _ag2347_a1		2.2x4tm6421f _ag2347_a1
Normal Colon GENPAK 061003	0.0	83793 Kidney NAT (OD04348)	0.0
97759 Colon cancer (OD06064)	0.0	98938 Kidney malignant cancer (OD06204B)	0.0
97760 Colon cancer NAT (OD06064)	0.0	98939 Kidney normal adjacent tissue (OD06204E)	0.0
97778 Colon cancer (OD06159)	0.0	85973 Kidney Cancer (OD04450-01)	0.0
97779 Colon cancer NAT (OD06159)	0.0	85974 Kidney NAT (OD04450-03)	0.0
98861 Colon cancer (OD06297-04)	0.0	Kidney Cancer Clontech 8120613	0.0
98862 Colon cancer NAT (OD06297-015)	0.0	Kidney NAT Clontech 8120614	0.0
83237 CC Gr.2 ascend colon	100.0	Kidney Cancer Clontech	0.0

<u>(ODO3921)</u>		9010320	
83238 CC NAT (ODO3921)	0.0	Kidney NAT Clontech 9010321	0.0
97766 Colon cancer metastasis (OD06104)	0.0	Kidney Cancer Clontech 8120607	0.0
97767 Lung NAT (OD06104)	0.0	Kidney NAT Clontech 8120608	0.0
87472 Colon mets to lung (OD04451-01)	0.0	Normal Uterus GENPAK 061018	0.0
87473 Lung NAT (OD04451-02)	0.0	Uterus Cancer GENPAK 064011	0.0
Normal Prostate Clontech A+ 6546-1 (8090438)	0.0	Normal Thyroid Clontech A+ 6570-1 (7080817)	0.0
84140 Prostate Cancer (OD04410)	0.5	Thyroid Cancer GENPAK 064010	0.0
84141 Prostate NAT (OD04410)	0.9	Thyroid Cancer INVITROGEN A302152	0.0
Normal Ovary Res. Gen.	0.0	Thyroid NAT INVITROGEN A302153	0.8
98863 Ovarian cancer (OD06283-03)	0.0	Normal Breast GENPAK 061019	0.4
98865 Ovarian cancer NAT/fallopian tube (OD06283-07)	0.0	84877 Breast Cancer (OD04566)	0.0
Ovarian Cancer GENPAK 064008	0.6	Breast Cancer Res. Gen. 1024	0.0
97773 Ovarian cancer (OD06145)	0.0	85975 Breast Cancer (OD04590-01)	0.0
97775 Ovarian cancer NAT (OD06145)	0.0	85976 Breast Cancer Mets (OD04590-03)	0.0
98853 Ovarian cancer (OD06455-03)	0.0	87070 Breast Cancer Metastasis (OD04655-05)	0.0
98854 Ovarian NAT (OD06455-07) Fallopian tube	0.0	GENPAK Breast Cancer 064006	0.0
Normal Lung GENPAK 061010	0.0	Breast Cancer Clontech 9100266	0.0
92337 Invasive poor diff. lung adeno (ODO4945-01)	0.0	Breast NAT Clontech 9100265	0.0
92338 Lung NAT (ODO4945-03)	0.0	Breast Cancer INVITROGEN A209073	0.0
84136 Lung Malignant Cancer (OD03126)	0.0	Breast NAT INVITROGEN A2090734	0.0
84137 Lung NAT (OD03126)	0.0	97763 Breast cancer (OD06083)	0.0
90372 Lung Cancer (OD05014A)	0.0	97764 Breast cancer node metastasis (OD06083)	0.0
90373 Lung NAT (OD05014B)	0.0	Normal Liver GENPAK 061009	0.0
97761 Lung cancer (OD06081)	0.0	Liver Cancer Research Genetics RNA 1026	0.0
97762 Lung cancer NAT	0.0	Liver Cancer Research Genetics	0.0

(OD06081)		RNA 1025	
85950 Lung Cancer (OD04237-01)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.0
85970 Lung NAT (OD04237-02)	0.0	Paired Liver Tissue Research Genetics RNA 6004-N	0.0
83255 Ocular Mel Met to Liver (ODO4310)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0
83256 Liver NAT (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
84139 Melanoma Mets to Lung (OD04321)	0.0	Liver Cancer GENPAK 064003	0.0
84138 Lung NAT (OD04321)	0.0	Normal Bladder GENPAK 061001	0.0
Normal Kidney GENPAK 061008	0.0	Bladder Cancer Research Genetics RNA 1023	0.0
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	Bladder Cancer INVITROGEN A302173	0.0
83787 Kidney NAT (OD04338)	0.0	Normal Stomach GENPAK 061017	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Gastric Cancer Clontech 9060397	0.0
83789 Kidney NAT (OD04339)	0.0	NAT Stomach Clontech 9060396	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	Gastric Cancer Clontech 9060395	0.0
83791 Kidney NAT (OD04340)	0.0	NAT Stomach Clontech 9060394	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Gastric Cancer GENPAK 064005	0.0

Table 24E. Panel 2D

Tissue Name	Relative Expression(%) 2dx4tm4693f_ag2482_b1	Tissue Name	Relative Expression(%) 2dx4tm4693f_ag2482_b1
Normal Colon GENPAK 061003	8.4	Kidney NAT Clontech 8120608	0.0
83219 CC Well to Mod Diff (ODO3866)	7.4	Kidney Cancer Clontech 8120613	0.0
83220 CC NAT (ODO3866)	6.7	Kidney NAT Clontech 8120614	0.0
83221 CC Gr.2 rectosigmoid (ODO3868)	2.5	Kidney Cancer Clontech 9010320	0.0
83222 CC NAT (ODO3868)	0.0	Kidney NAT Clontech 9010321	0.0
83235 CC Mod Diff (ODO3920)	0.0	Normal Uterus GENPAK 061018	0.0
83236 CC NAT (ODO3920)	0.0	Uterus Cancer GENPAK 064011	0.0

83237 CC Gr.2 ascend colon (ODO3921)	0.0	Normal Thyroid Clontech A+ 6570-1	0.0
83238 CC NAT (ODO3921)	4.6	Thyroid Cancer GENPAK 064010	0.0
83241 CC from Partial Hepatectomy (ODO4309)	0.0	Thyroid Cancer INVITROGEN A302152	0.0
83242 Liver NAT (ODO4309)	0.0	Thyroid NAT INVITROGEN A302153	0.0
87472 Colon mets to lung (OD04451-01)	0.0	Normal Breast GENPAK 061019	0.0
87473 Lung NAT (OD04451-02)	0.0	84877 Breast Cancer (OD04566)	0.0
Normal Prostate Clontech A+ 6546-1	32.7	85975 Breast Cancer (OD04590-01)	0.0
84140 Prostate Cancer (OD04410)	100.0	85976 Breast Cancer Mets (OD04590-03)	0.0
84141 Prostate NAT (OD04410)	44.7	87070 Breast Cancer Metastasis (OD04655-05)	7.2
87073 Prostate Cancer (OD04720-01)	9.7	GENPAK Breast Cancer 064006	0.0
87074 Prostate NAT (OD04720-02)	62.0	Breast Cancer Res. Gen. 1024	0.0
Normal Lung GENPAK 061010	0.0	Breast Cancer Clontech 9100266	0.0
83239 Lung Met to Muscle (ODO4286)	12.2	Breast NAT Clontech 9100265	0.0
83240 Muscle NAT (ODO4286)	0.0	Breast Cancer INVITROGEN A209073	0.0
84136 Lung Malignant Cancer (OD03126)	25.5	Breast NAT INVITROGEN A2090734	0.0
84137 Lung NAT (OD03126)	0.0	Normal Liver GENPAK 061009	0.0
84871 Lung Cancer (OD04404)	0.0	Liver Cancer GENPAK 064003	0.0
84872 Lung NAT (OD04404)	0.0	Liver Cancer Research Genetics RNA 1025	0.0
84875 Lung Cancer (OD04565)	0.0	Liver Cancer Research Genetics RNA 1026	0.0
84876 Lung NAT (OD04565)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	2.4
85950 Lung Cancer (OD04237-01)	0.0	Paired Liver Tissue Research Genetics RNA 6004-N	0.0
85970 Lung NAT (OD04237-02)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0
83255 Ocular Mel Met to Liver (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
83256 Liver NAT (ODO4310)	0.0	Normal Bladder GENPAK 061001	2.1

84139 Melanoma Mets to Lung (OD04321)	0.0	Bladder Cancer Research Genetics RNA 1023	0.0
84138 Lung NAT (OD04321)	0.0	Bladder Cancer INVITROGEN A302173	18.7
Normal Kidney GENPAK 061008	0.0	87071 Bladder Cancer (OD04718-01)	0.0
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	87072 Bladder Normal Adjacent (OD04718-03)	0.0
83787 Kidney NAT (OD04338)	3.3	Normal Ovary Res. Gen.	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Ovarian Cancer GENPAK 064008	0.0
83789 Kidney NAT (OD04339)	0.0	87492 Ovary Cancer (OD04768-07)	0.0
83790 Kidney Ca, Clear cell type (OD04340)	5.1	87493 Ovary NAT (OD04768-08)	0.0
83791 Kidney NAT (OD04340)	0.0	Normal Stomach GENPAK 061017	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Gastric Cancer Clontech 9060358	0.0
83793 Kidney NAT (OD04348)	0.0	NAT Stomach Clontech 9060359	0.0
87474 Kidney Cancer (OD04622-01)	0.0	Gastric Cancer Clontech 9060395	0.0
87475 Kidney NAT (OD04622-03)	0.0	NAT Stomach Clontech 9060394	0.0
85973 Kidney Cancer (OD04450-01)	0.0	Gastric Cancer Clontech 9060397	2.7
85974 Kidney NAT (OD04450-03)	0.0	NAT Stomach Clontech 9060396	0.0
Kidney Cancer Clontech 8120607	0.0	Gastric Cancer GENPAK 064005	0.0

Table 24F. Panel 3D

Tissue Name	Relative Expression(%) 3dx4tm5085f_ ag2482_b2	Tissue Name	Relative Expression(%) 3dx4tm5085f_ ag2482_b2
94905_Daoy_Medulloblastoma/Cerebellum_sscDNA	0.0	94954_Ca_Ski_Cervical epidermoid carcinoma (metastasis)_sscDNA	0.0
94906_TE671_Medulloblastom/Cerebellum_sscDNA	0.0	94955_ES-2_Ovarian clear cell carcinoma_sscDNA	0.0
94907_D283_Med_Medulloblastoma/Cerebellum_sscDNA	0.0	94957_Ramos/6h_stim_Stimulated with PMA/ionomycin 6h_sscDNA	0.0
94908_PFSK-1_Primitive Neuroectodermal/Cerebellum_sscDNA	0.0	94958_Ramos/14h_stim_Stimulated with PMA/ionomycin 14h_sscDNA	0.0
94909_XF-498_CNS_sscDNA	0.0	94962_MEG-01_Chronic myelogenous leukemia	0.0

		(megakaryoblast)_sscDNA	
94910_SNB-78_CNS/glioma_sscDNA	0.0	94963_Raji_Burkitt's lymphoma_sscDNA	0.0
94911_SF-268_CNS/glioblastoma_sscDNA	0.0	94964_Daudi_Burkitt's lymphoma_sscDNA	0.0
94912_T98G_Glioblastoma_sscDNA	0.0	94965_U266_B-cell plasmacytoma/myeloma_sscDNA	0.0
96776_SK-N-SH_Neuroblastoma (metastasis)_sscDNA	0.0	94968_CA46_Burkitt's lymphoma_sscDNA	0.0
94913_SF-295_CNS/glioblastoma_sscDNA	0.0	94970_RL_non-Hodgkin's B-cell lymphoma_sscDNA	0.0
94914_Cerebellum_sscDNA	0.0	94972_JM1_pre-B-cell lymphoma/leukemia_sscDNA	0.0
96777_Cerebellum_sscDNA	0.0	94973_Jurkat_T cell leukemia_sscDNA	0.0
94916_NCI-H292_Mucoepidermoid lung carcinoma_sscDNA	0.0	94974_TF-1_Erythroleukemia_sscDNA	0.0
94917_DMS-114_Small cell lung cancer_sscDNA	0.0	94975_HUT 78_T-cell lymphoma_sscDNA	0.0
94918_DMS-79_Small cell lung cancer/neuroendocrine_sscDNA	100.0	94977_U937_Histiocytic lymphoma_sscDNA	0.0
94919_NCI-H146_Small cell lung cancer/neuroendocrine_sscDNA	12.7	94980_KU-812_Myelogenous leukemia_sscDNA	0.0
94920_NCI-H526_Small cell lung cancer/neuroendocrine_sscDNA	4.4	94981_769-P_Clear cell renal carcinoma_sscDNA	0.0
94921_NCI-N417_Small cell lung cancer/neuroendocrine_sscDNA	0.0	94983_Caki-2_Clear cell renal carcinoma_sscDNA	0.0
94923_NCI-H82_Small cell lung cancer/neuroendocrine_sscDNA	0.0	94984_SW 839_Clear cell renal carcinoma_sscDNA	0.0
94924_NCI-H157_Squamous cell lung cancer (metastasis)_sscDNA	0.0	94986_G401_Wilms' tumor_sscDNA	0.0
94925_NCI-H1155_Large cell lung cancer/neuroendocrine_sscDNA	0.0	94987_Hs766T_Pancreatic carcinoma (LN metastasis)_sscDNA	0.0
94926_NCI-H1299_Large cell lung cancer/neuroendocrine_sscDNA	0.0	94988_CAPAN-1_Pancreatic adenocarcinoma (liver metastasis)_sscDNA	0.0
94927_NCI-H727_Lung carcinoid_sscDNA	0.0	94989_SU86.86_Pancreatic carcinoma (liver	0.0

		metastasis)_sscDNA	
94928_NCI-UMC-11_Lung carcinoid_sscDNA	8.8	94990_BxPC-3_Pancreatic adenocarcinoma_sscDNA	0.0
94929_LX-1_Small cell lung cancer_sscDNA	0.0	94991_HPAC_Pancreatic adenocarcinoma_sscDNA	0.0
94930_Colo-205_Colon cancer_sscDNA	0.0	94992_MIA PaCa-2_Pancreatic carcinoma_sscDNA	0.0
94931_KM12_Colon cancer_sscDNA	0.0	94993_CFPAC-1_Pancreatic ductal adenocarcinoma_sscDNA	0.0
94932_KM20L2_Colon cancer_sscDNA	0.0	94994_PANC-1_Pancreatic epithelioid ductal carcinoma_sscDNA	0.0
94933_NCI-H716_Colon cancer_sscDNA	0.0	94996_T24_Bladder carcinoma (transitional cell)_sscDNA	0.0
94935_SW-48_Colon adenocarcinoma_sscDNA	0.0	94997_5637_Bladder carcinoma_sscDNA	0.0
94936_SW1116_Colon adenocarcinoma_sscDNA	0.0	94998_HT-1197_Bladder carcinoma_sscDNA	0.0
94937_LS 174T_Colon adenocarcinoma_sscDNA	0.0	94999_UM-UC-3_Bladder carcinoma (transitional cell)_sscDNA	0.0
94938_SW-948_Colon adenocarcinoma_sscDNA	0.0	95000_A204_Rhabdomyosarcoma sscDNA	0.0
94939_SW-480_Colon adenocarcinoma_sscDNA	0.0	95001_HT- 1080_Fibrosarcoma_sscDNA	0.0
94940_NCI-SNU-5_Gastric carcinoma_sscDNA	0.0	95002_MG-63_Osteosarcoma (bone)_sscDNA	8.5
94941_KATO III_Gastric carcinoma_sscDNA	0.0	95003_SK-LMS- 1_Leiomyosarcoma (vulva)_sscDNA	0.0
94943_NCI-SNU-16_Gastric carcinoma_sscDNA	0.0	95004_SJRH30_Rhabdomyosarcoma (met to bone marrow)_sscDNA	0.0
94944_NCI-SNU-1_Gastric carcinoma_sscDNA	0.0	95005_A431_Epidermoid carcinoma_sscDNA	0.0
94946_RF-1_Gastric adenocarcinoma_sscDNA	0.0	95007_WM266- 4_Melanoma_sscDNA	0.0
94947_RF-48_Gastric adenocarcinoma_sscDNA	0.0	95010_DU 145_Prostate carcinoma (brain metastasis)_sscDNA	0.0
96778_MKN-45_Gastric carcinoma_sscDNA	0.0	95012_MDA-MB-468_Breast adenocarcinoma_sscDNA	0.0
94949_NCI-N87_Gastric carcinoma_sscDNA	0.0	95013_SCC-4_Squamous cell carcinoma of tongue_sscDNA	0.0
94951_OVCAR-5_Ovarian carcinoma_sscDNA	0.0	95014_SCC-9_Squamous cell carcinoma of tongue_sscDNA	0.0
94952_RL95-2_Uterine carcinoma_sscDNA	0.0	95015_SCC-15_Squamous cell carcinoma of tongue_sscDNA	0.0

94953_HelaS3_Cervical adenocarcinoma_sscDNA	0.0	95017_CAL 27_Squamous cell carcinoma of tongue_sscDNA	0.0
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Panel 1.3D Summary Ag2482 Expression of the CG50197-01 gene in two independent runs is highest in a sample derived from a lung cancer cell line (SHP-77). Its expression in this panel is almost exclusive to this sample. Thus, the expression the CG50197-01 gene could be used to distinguish samples derived from this cell line and other samples. Furthermore, therapeutic modulation of the expression or function of the protein encoded by the CG50197-01 gene, through the use of small molecule drugs or antibodies, may be useful in the treatment of lung cancer. **Ag2347** Expression of the CG50197-01 gene in panel 1.3D is low/undetectable (CT values >35) in all samples (data not shown).

Panel 2.2 Summary Ag2347 Expression of the CG50197-01 gene is limited to a sample derived from a colon cancer (CT=31.4). This result suggests that expression of the CG50197-01 gene could be used as a diagnostic marker for the presence of colon cancer. Furthermore, therapeutic modulation of the expression or function of the CG50197-01 gene product, through the use of small molecule drugs or antibodies, may be effective in the treatment of colon cancer. **Ag2482** Expression of the CG50197-01 gene in Panel 2.2 is low/undetectable (CT values >35) in all samples (data not shown).

Panel 2D Summary Ag2482 Expression of the CG50197-01 gene is highest in a sample derived from a prostate cancer and overall, its expression appears to be specific for prostate tissue. In addition, one sample derived from prostate cancer shows substantial over expression when compared to a matched sample derived from normal adjacent tissue. Thus, the expression of this gene could be used to distinguish prostate derived tissue from other tissues. Moreover, therapeutic modulation of the expression or function of the CG50197-01 gene or its protein product, through the use of small molecule drugs, antibodies or protein therapeutics, might be of use in the treatment of prostate cancer.

Panel 3D Summary Ag2482 Significant expression of the CG50197-01 gene is limited to a sample derived from a lung cancer cell line. This preferential expression in lung cancer is also seen in the expression profiles from Panel 1.3D. This result suggests that expression of the CG50197-01 gene could be used to distinguish this cell line from other samples. Furthermore, therapeutic modulation of the gene or its protein product could potentially be useful in the treatment of lung cancer.

Panel 4D Summary Ag2482/Ag2347 Expression of the CG50197-01 gene in this panel is low/undetectable (CT values >35) in all samples (data not shown).

Panel CNS_neurodegeneration_v1.0 Ag2347 Expression of the CG50197-01 gene in panel CNS_neurodegeneration_v1.0 is low/undetectable (CT values >35) in all samples (data not shown).

GPCR11 (also referred to as CG50199-01)

Expression of gene CG50199-01 was assessed using the primer-probe sets Ag2483 and Ag1729, described in Tables 25A and 25B. Results from RTQ-PCR runs are shown in Tables 25C, 25D, and 25E.

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Table 25A Probe Name Ag2483 (SEQ ID NO: 198, 199, 200)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GTGAATTTGTTCTCGTGAGCTT-3'	58.5	22	3
Probe	FAM-5'-CCCTGTCCACTGAGCTTCAGGCTCTA-3'-TAMRA	69.8	26	30
Reverse	5'-TGGTCAAGAAAAGGAGAAACAG-3'	58.5	22	56

Table 25B. Probe Name Ag1729 (SEQ ID NO: 201, 202, 203)

Primers	Sequences	TM	Length	Start Position
Forward	5'-ATTGTTTCTCGTGAGCTTCTCA-3'	59.1	22	34
Probe	TET-5'-CCCTGTCCACTGAGCTTCAGGCTCTA-3'-TAMRA	69.8	26	57
Reverse	5'-CATTGCCCATTAAAGTAACCAA-3'	58.9	22	110

Table 25C. Panel 1.3D

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	1.3dx4tm5458 f_ag2483_a1		1.3dx4tm5458 f_ag2483_a1
Liver adenocarcinoma	0.0	Kidney (fetal)	0.0
Pancreas	65.3	Renal ca. 786-0	34.4
Pancreatic ca. CAPAN 2	0.0	Renal ca. A498	18.1
Adrenal gland	37.1	Renal ca. RXF 393	0.0
Thyroid	0.0	Renal ca. ACHN	0.0
Salivary gland	0.0	Renal ca. UO-31	0.0
Pituitary gland	0.0	Renal ca. TK-10	0.0
Brain (fetal)	19.4	Liver	0.0
Brain (whole)	74.3	Liver (fetal)	0.0
Brain (amygdala)	12.9	Liver ca. (hepatoblast) HepG2	0.0
Brain (cerebellum)	39.9	Lung	55.1
Brain (hippocampus)	10.0	Lung (fetal)	0.0
Brain (substantia nigra)	50.0	Lung ca. (small cell) LX-1	86.5
Brain (thalamus)	18.5	Lung ca. (small cell) NCI-H69	0.0
Cerebral Cortex	37.2	Lung ca. (s.cell var.) SHP-77	0.0

Spinal cord	0.0	Lung ca. (large cell)NCI-H460	17.6
CNS ca. (glio/astro) U87-MG	36.8	Lung ca. (non-sm. cell) A549	0.0
CNS ca. (glio/astro) U-118-MG	37.5	Lung ca. (non-s.cell) NCI-H23	46.0
CNS ca. (astro) SW1783	69.8	Lung ca (non-s.cell) HOP-62	0.0
CNS ca.* (neuro; met) SK-N-AS	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
CNS ca. (astro) SF-539	0.0	Lung ca. (squam.) SW 900	22.2
CNS ca. (astro) SNB-75	44.1	Lung ca. (squam.) NCI-H596	0.0
CNS ca. (glio) SNB-19	0.0	Mammary gland	39.0
CNS ca. (glio) U251	34.9	Breast ca.* (pl. effusion) MCF-7	0.0
CNS ca. (glio) SF-295	53.3	Breast ca.* (pl.ef) MDA-MB-231	100.0
Heart (fetal)	0.0	Breast ca.* (pl. effusion) T47D	0.0
Heart	41.8	Breast ca. BT-549	0.0
Fetal Skeletal	0.0	Breast ca. MDA-N	15.0
Skeletal muscle	0.0	Ovary	0.0
Bone marrow	14.7	Ovarian ca. OVCAR-3	0.0
Thymus	33.0	Ovarian ca. OVCAR-4	0.0
Spleen	0.0	Ovarian ca. OVCAR-5	21.2
Lymph node	83.2	Ovarian ca. OVCAR-8	31.4
Colorectal	32.1	Ovarian ca. IGROV-1	0.0
Stomach	21.5	Ovarian ca.* (ascites) SK-OV-3	52.5
Small intestine	45.2	Uterus	4.9
Colon ca. SW480	57.2	Placenta	83.8
Colon ca.* (SW480 met)SW620	37.3	Prostate	0.0
Colon ca. HT29	22.1	Prostate ca.* (bone met)PC-3	17.1
Colon ca. HCT-116	45.8	Testis	80.4
Colon ca. CaCo-2	0.0	Melanoma Hs688(A).T	0.0
83219 CC Well to Mod Diff (ODO3866)	7.1	Melanoma* (met) Hs688(B).T	0.0
Colon ca. HCC-2998	0.0	Melanoma UACC-62	40.0
Gastric ca.* (liver met) NCI-N87	0.0	Melanoma M14	13.7
Bladder	47.2	Melanoma LOX IMVI	0.0
Trachea	20.1	Melanoma* (met) SK-MEL-5	0.0
Kidney	0.0	Adipose	0.0

Table 25D. Panel 4D

Tissue Name	Relative Expression(%)	Relative Expression(%)
	4dx4tm5031f_ag2483_b2	4dx4tm5529t_ag1729_b2

93768 Secondary Th1 anti-CD28/anti-CD3	7.1	11.8
93769 Secondary Th2 anti-CD28/anti-CD3	30.8	97.4
93770 Secondary Tr1 anti-CD28/anti-CD3	19.7	100.0
93573 Secondary Th1 resting day 4-6 in IL-2	0.0	5.4
93572 Secondary Th2 resting day 4-6 in IL-2	3.2	4.0
93571 Secondary Tr1 resting day 4-6 in IL-2	1.5	7.4
93568 primary Th1 anti-CD28/anti-CD3	18.6	40.9
93569 primary Th2 anti-CD28/anti-CD3	7.9	47.8
93570 primary Tr1 anti-CD28/anti-CD3	13.9	91.3
93565 primary Th1 resting dy 4-6 in IL-2	9.4	28.8
93566 primary Th2 resting dy 4-6 in IL-2	2.2	12.3
93567 primary Tr1 resting dy 4-6 in IL-2	5.3	13.4
93351 CD45RA CD4 lymphocyte anti-CD28/anti-CD3	2.5	8.8
93352 CD45RO CD4 lymphocyte anti-CD28/anti-CD3	7.3	17.4
93251 CD8 Lymphocytes anti-CD28/anti-CD3	3.8	10.4
93353 chronic CD8 Lymphocytes 2ry resting dy 4-6 in IL-2	1.9	5.2
93574 chronic CD8 Lymphocytes 2ry activated CD3/CD28	5.5	13.3
93354 CD4 none	0.5	0.8
93252 Secondary Th1/Th2/Tr1 anti-CD95 CH11	3.4	8.4
93103 LAK cells resting	1.6	1.7
93788 LAK cells IL-2	1.0	3.4
93787 LAK cells IL-2+IL-12	0.7	3.9
93789 LAK cells IL-2+IFN gamma	0.9	5.3
93790 LAK cells IL-2+ IL-18	1.2	0.6
93104 LAK cells PMA/ionomycin and IL-18	1.7	4.2
93578 NK Cells IL-2 resting	0.4	1.5
93109 Mixed Lymphocyte Reaction Two Way MLR	0.6	0.0
93110 Mixed Lymphocyte Reaction Two Way MLR	1.0	2.4
93111 Mixed Lymphocyte Reaction Two Way MLR	1.5	5.0
93112 Mononuclear Cells (PBMCs) resting	0.9	2.7
93113 Mononuclear Cells (PBMCs) PWM	3.1	0.6
93114 Mononuclear Cells (PBMCs) PHA-L	8.2	5.2
93249 Ramos (B cell) none	33.5	94.1
93250 Ramos (B cell) ionomycin	100.0	72.3
93349 B lymphocytes PWM	2.1	0.8
93350 B lymphocytes CD40L and IL-4	0.9	3.6
92665 EOL-1 (Eosinophil) dbcAMP differentiated	0.0	0.0
93248 EOL-1 (Eosinophil) dbcAMP/PMAionomycin	0.0	0.0
93356 Dendritic Cells none	0.6	0.0
93355 Dendritic Cells LPS 100 ng/ml	0.0	0.0
93775 Dendritic Cells anti-CD40	1.0	0.0
93774 Monocytes resting	0.0	0.0

93776_Monocytes_LPS 50 ng/ml	0.0	0.0
93581_Macrophages_resting	4.0	11.5
93582_Macrophages_LPS 100 ng/ml	0.0	0.7
93098_HUVEC (Endothelial)_none	6.9	25.9
93099_HUVEC (Endothelial)_starved	15.1	55.5
93100_HUVEC (Endothelial)_IL-1b	2.4	20.2
93779_HUVEC (Endothelial)_IFN gamma	6.8	14.2
93102_HUVEC (Endothelial)_TNF alpha + IFN gamma	5.1	4.8
93101_HUVEC (Endothelial)_TNF alpha + IL4	7.8	11.9
93781_HUVEC (Endothelial)_IL-11	1.9	8.5
93583_Lung Microvascular Endothelial Cells_none	9.6	13.3
93584_Lung Microvascular Endothelial Cells_TNFa (4 ng/ml) and IL1b (1 ng/ml)	6.9	9.7
92662_Microvascular Dermal endothelium_none	1.2	3.6
92663_Microvascular Dermal endothelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	1.0	3.8
93773_Bronchial epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)**	1.9	2.2
93347_Small Airway Epithelium_none	0.7	0.0
93348_Small Airway Epithelium_TNFa (4 ng/ml) and IL1b (1 ng/ml)	15.1	18.8
92668_Coronary Artery SMC_resting	3.0	8.4
92669_Coronary Artery SMC_TNFa (4 ng/ml) and IL1b (1 ng/ml)	3.7	3.2
93107_astrocytes_resting	0.0	5.1
93108_astrocytes_TNFa (4 ng/ml) and IL1b (1 ng/ml)	1.9	6.5
92666_KU-812 (Basophil)_resting	2.7	10.1
92667_KU-812 (Basophil)_PMA/ionoycin	11.2	26.6
93579_CCD1106 (Keratinocytes)_none	7.5	7.4
93580_CCD1106 (Keratinocytes)_TNFa and IFNg **	0.0	37.3
93791_Liver Cirrhosis	4.8	29.7
93792_Lupus Kidney	1.7	3.3
93577_NCI-H292	25.2	54.5
93358_NCI-H292_IL-4	23.4	55.7
93360_NCI-H292_IL-9	22.2	65.5
93359_NCI-H292_IL-13	8.3	31.9
93357_NCI-H292_IFN gamma	15.0	29.2
93777_HPAEC_-	6.2	11.0
93778_HPAEC_IL-1 beta/TNA alpha	7.5	18.1
93254_Normal Human Lung Fibroblast_none	3.0	6.2
93253_Normal Human Lung Fibroblast_TNFa (4 ng/ml) and IL-1b (1 ng/ml)	1.1	8.7
93257_Normal Human Lung Fibroblast_IL-4	1.9	2.8
93256_Normal Human Lung Fibroblast_IL-9	4.0	9.9

93255_Normal Human Lung Fibroblast_IL-13	1.4	8.9
93258_Normal Human Lung Fibroblast_IFN gamma	1.9	6.2
93106_Dermal Fibroblasts CCD1070_resting	14.5	57.5
93361_Dermal Fibroblasts CCD1070_TNF alpha 4 ng/ml	30.6	52.4
93105_Dermal Fibroblasts CCD1070_IL-1 beta 1 ng/ml	12.3	20.9
93772_dermal fibroblast_IFN gamma	1.9	0.5
93771_dermal fibroblast_IL-4	1.6	0.7
93260_IBD Colitis 2	2.6	3.5
93261_IBD Crohns	0.8	2.5
735010_Colon_normal	6.2	19.7
735019_Lung_none	0.9	1.7
64028-1_Thymus_none	7.3	9.0
64030-1_Kidney_none	7.8	8.0

Table 25E. Panel CNS_neurodegeneration_v1.0

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	tm7041f_ag2483_b1		tm7041f_ag2483_b1
AD 1 Hippo	9.4	Control (Path) 3 Temporal Ctx	10.6
AD 2 Hippo	15.1	Control (Path) 4 Temporal Ctx	20.8
AD 3 Hippo	5.0	AD 1 Occipital Ctx	15.3
AD 4 Hippo	23.1	AD 2 Occipital Ctx (Missing)	0.0
AD 5 Hippo	90.6	AD 3 Occipital Ctx	0.0
AD 6 Hippo	23.2	AD 4 Occipital Ctx	14.9
Control 2 Hippo	12.2	AD 5 Occipital Ctx	11.2
Control 4 Hippo	2.5	AD 6 Occipital Ctx	11.7
Control (Path) 3 Hippo	14.0	Control 1 Occipital Ctx	2.1
AD 1 Temporal Ctx	2.9	Control 2 Occipital Ctx	22.9
AD 2 Temporal Ctx	18.5	Control 3 Occipital Ctx	30.1
AD 3 Temporal Ctx	0.0	Control 4 Occipital Ctx	0.0
AD 4 Temporal Ctx	17.9	Control (Path) 1 Occipital Ctx	84.1
AD 5 Inf Temporal Ctx	39.8	Control (Path) 2 Occipital Ctx	29.6
AD 5 Sup Temporal Ctx	21.9	Control (Path) 3 Occipital Ctx	0.0
AD 6 Inf Temporal Ctx	14.8	Control (Path) 4 Occipital Ctx	50.7
AD 6 Sup Temporal Ctx	22.2	Control 1 Parietal Ctx	13.7
Control 1 Temporal Ctx	9.3	Control 2 Parietal Ctx	32.1
Control 2 Temporal Ctx	9.8	Control 3 Parietal Ctx	16.6
Control 3 Temporal Ctx	20.2	Control (Path) 1 Parietal Ctx	70.2
Control 3 Temporal Ctx	18.7	Control (Path) 2 Parietal Ctx	36.6
Control (Path) 1 Temporal Ctx	100.0	Control (Path) 3 Parietal Ctx	8.0
Control (Path) 2 Temporal Ctx	78.6	Control (Path) 4 Parietal Ctx	86.0

Panel 1.3D Summary Ag2483 Significant expression of the CG50199-01 gene is limited to a sample derived from a breast cancer (CT=34.9). Thus, the expression of this gene could be used to distinguish this cell line from other samples. Furthermore, therapeutic modulation of the expression or function of the protein encoded by the CG50199-01 gene, through the use of small molecule drugs or antibodies, might be beneficial in the treatment of breast cancer.

Panel 4D Summary Ag2483/Ag1729 Two experiments using two different probe and primer sets show highest expression of the CG50199-01 gene in ionomycin-activated Ramos B lymphblastoid cells (CT=29.4) in one run and in activated Tr1 cells (CT=32.2) in the second run. Moderate expression is also detected in activated Th1 and Th2 cells, resting and cytokine-activated dermal fibroblasts, and in resting and cytokine activated mucoepidermoid cells. Inhibition of the function of the protein encoded by the CG50199-01 gene by a specific antibody or small molecule drug may reduce inflammation and autoimmunity that result from asthma, psoriasis, and inflammatory bowel disease.

Panel CNS_neurodegeneration_v1.0 Summary Ag2483 The protein encoded by the CG50199-01 gene contains homology to the GPCR family of receptors, and is shown by panel CNS_Neurodegeneration_V1.0 to be expressed in the brain, with gene expression down regulated in the temporal cortex of the Alzheimer's diseased brain. The temporal cortex is a region that specifically shows neurodegeneration in Alzheimer's disease. Several neurotransmitter receptors are GPCRs, including the dopamine receptor family, the serotonin receptor family, the GABAB receptor, and the muscarinic acetylcholine receptors. Thus, the GPCR encoded by the CG50199-01 gene may represent a novel neurotransmitter receptor. Targeting various neurotransmitter receptors, such as dopamine, serotonin receptors, has proven to be an effective therapy in psychiatric illnesses such as schizophrenia, bipolar disorder and depression. Furthermore the cerebral cortex and hippocampus are regions of the brain that are known to play critical roles in Alzheimer's disease, seizure disorders, and in the normal process of memory formation. Thus, therapeutic modulation of this gene or its protein product may be beneficial in one or more of these diseases, as may stimulation of the receptor coded for by the gene.

GPCR12 (also referred to as CG50217-01)

Expression of gene CG50217-01 was assessed using the primer-probe sets Ag2494 and Ag2345, described in Tables 26A and 26B. Results from RTQ-PCR runs are shown in Tables 26C and 26D.

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Table 26A Probe Name Ag2494 (SEQ ID NO: 204, 205, 206)

Primers	Sequences	TM	Length	Start Position
Forward	5'-TTCCACAACATCCTTGGATAAC-3'	58.8	22	86
Probe	TET-5'-CCCCGATCTGTTTGGTTCTAACTCCA-3'-TAMRA	68.9	26	113
Reverse	5'-TACTGATACCTCCCATGCTCAA-3'	59.6	22	151

Table 26B. Probe Name Ag2345 (contains a single base mismatch within the probe sequence) (SEQ ID NO: 207, 208, 209)

Primers	Sequences	TM	Length	Start Position
Forward	5'-GATTTTGAGAGCTGTGCTTCAG-3'	59.3	22	672
Probe	FAM-5'-CCTCAAAGCTTTTAGCACACGTGCCT-3'-TAMRA	69.2	26	714
Reverse	5'-AGCCAAGATGACACAGATATGG-3'	59.1	22	741

Table 26C. Panel 1.3D

Tissue Name	Relative Expression(%)	Relative Expression(%)
	1.3dx4tm5465 t ag2494 b2	1.3dx4tm5618 f ag2345 b1
Liver adenocarcinoma	0.0	0.0
Pancreas	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0
Adrenal gland	7.4	0.0
Thyroid	0.0	0.0
Salivary gland	0.0	0.0
Pituitary gland	0.0	0.0
Brain (fetal)	0.0	0.0
Brain (whole)	0.0	0.0
Brain (amygdala)	0.0	0.0
Brain (cerebellum)	0.0	0.0
Brain (hippocampus)	0.0	0.0
Brain (substantia nigra)	17.9	0.0

Brain (thalamus)	0.0	0.0
Cerebral Cortex	0.0	0.0
Spinal cord	21.3	0.0
CNS ca. (glio/astro) U87-MG	0.0	0.0
CNS ca. (glio/astro) U-118-MG	0.0	0.0
CNS ca. (astro) SW1783	0.0	4.8
CNS ca.* (neuro; met) SK-N-AS	0.0	0.0
CNS ca. (astro) SF-539	0.0	0.0
CNS ca. (astro) SNB-75	0.0	0.0
CNS ca. (glio) SNB-19	0.0	2.3
CNS ca. (glio) U251	0.0	0.0
CNS ca. (glio) SF-295	0.0	0.0
Heart (fetal)	0.0	0.0
Heart	0.0	0.0
Fetal Skeletal	0.0	0.0
Skeletal muscle	0.0	0.0
Bone marrow	34.1	0.0
Thymus	0.0	0.0
Spleen	0.0	0.0
Lymph node	4.5	0.0
Colorectal	7.0	0.0
Stomach	0.0	0.0
Small intestine	0.0	0.0
Colon ca. SW480	0.0	0.0
Colon ca.* (SW480 met)SW620	0.0	0.0
Colon ca. HT29	15.6	0.0
Colon ca. HCT-116	0.0	0.0
Colon ca. CaCo-2	0.0	0.0
<u>83219 CC Well to Mod Diff (ODO3866)</u>	14.2	0.0
Colon ca. HCC-2998	0.0	0.0
Gastric ca.* (liver met) NCI-N87	100.0	0.0
Bladder	49.3	0.8
Trachea	0.0	0.0
Kidney	0.0	0.0
Kidney (fetal)	0.0	0.0
Renal ca. 786-0	0.0	0.0
Renal ca. A498	0.0	1.4
Renal ca. RXF 393	0.0	0.0
Renal ca. ACHN	0.0	0.0
Renal ca. UO-31	0.0	0.0
Renal ca. TK-10	0.0	0.0
Liver	0.0	0.0

Liver (fetal)	0.0	0.0
Liver ca. (hepatoblast) HepG2	0.0	0.0
Lung	0.0	0.0
Lung (fetal)	0.0	0.0
Lung ca. (small cell) LX-1	0.0	0.0
Lung ca. (small cell) NCI-H69	0.0	48.4
Lung ca. (s.cell var.) SHP-77	0.0	100.0
Lung ca. (large cell) NCI-H460	0.0	0.0
Lung ca. (non-sm. cell) A549	0.0	0.0
Lung ca. (non-s.cell) NCI-H23	0.0	0.0
Lung ca (non-s.cell) HOP-62	0.0	0.0
Lung ca. (non-s.cl) NCI-H522	0.0	0.0
Lung ca. (squam.) SW 900	15.6	0.0
Lung ca. (squam.) NCI-H596	0.0	64.0
Mammary gland	0.0	0.0
Breast ca.* (pl. effusion) MCF-7	0.0	0.0
Breast ca.* (pl.ef) MDA-MB-231	15.5	0.0
Breast ca.* (pl. effusion) T47D	0.0	0.0
Breast ca. BT-549	0.0	0.0
Breast ca. MDA-N	0.0	0.0
Ovary	0.0	0.0
Ovarian ca. OVCAR-3	0.0	0.0
Ovarian ca. OVCAR-4	0.0	0.0
Ovarian ca. OVCAR-5	0.0	0.0
Ovarian ca. OVCAR-8	0.0	0.0
Ovarian ca. IGROV-1	0.0	4.8
Ovarian ca.* (ascites) SK-OV-3	0.0	0.0
Uterus	0.0	0.0
Placenta	13.0	0.0
Prostate	0.0	2.6
Prostate ca.* (bone met) PC-3	0.0	2.5
Testis	0.0	2.0
Melanoma Hs688(A).T	0.0	0.0
Melanoma* (met) Hs688(B).T	0.0	0.0
Melanoma UACC-62	0.0	0.0
Melanoma M14	0.0	0.0
Melanoma LOX IMVI	0.0	0.0
Melanoma* (met) SK-MEL-5	0.0	0.0
Adipose	0.0	0.0

Table 26D. Panel 2.2

Tissue Name	Relative Expression(%)	Tissue Name	Relative Expression(%)
	2.2x4tm6420f ag2345 b2		2.2x4tm6420f ag2345 b2
Normal Colon GENPAK 061003	5.3	83793 Kidney NAT (OD04348)	0.0
97759 Colon cancer (OD06064)	0.0	98938 Kidney malignant cancer (OD06204B)	0.0
97760 Colon cancer NAT (OD06064)	0.0	98939 Kidney normal adjacent tissue (OD06204E)	0.0
97778 Colon cancer (OD06159)	0.0	85973 Kidney Cancer (OD04450-01)	0.0
97779 Colon cancer NAT (OD06159)	0.0	85974 Kidney NAT (OD04450-03)	0.0
98861 Colon cancer (OD06297-04)	0.0	Kidney Cancer Clontech 8120613	0.0
98862 Colon cancer NAT (OD06297-015)	0.0	Kidney NAT Clontech 8120614	0.0
83237 CC Gr.2 ascend colon (ODO3921)	0.0	Kidney Cancer Clontech 9010320	0.0
83238 CC NAT (ODO3921)	0.0	Kidney NAT Clontech 9010321	0.0
97766 Colon cancer metastasis (OD06104)	0.0	Kidney Cancer Clontech 8120607	0.0
97767 Lung NAT (OD06104)	0.0	Kidney NAT Clontech 8120608	0.0
87472 Colon mets to lung (OD04451-01)	0.0	Normal Uterus GENPAK 061018	0.0
87473 Lung NAT (OD04451-02)	0.0	Uterus Cancer GENPAK 064011	6.8
Normal Prostate Clontech A+ 6546-1 (8090438)	18.2	Normal Thyroid Clontech A+ 6570-1 (7080817)	0.0
84140 Prostate Cancer (OD04410)	6.5	Thyroid Cancer GENPAK 064010	0.0
84141 Prostate NAT (OD04410)	100.0	Thyroid Cancer INVITROGEN A302152	0.0
Normal Ovary Res. Gen.	0.0	Thyroid NAT INVITROGEN A302153	3.1
98863 Ovarian cancer (OD06283-03)	0.0	Normal Breast GENPAK 061019	0.0
98865 Ovarian cancer NAT/fallopian tube (OD06283-07)	0.0	84877 Breast Cancer (OD04566)	0.0
Ovarian Cancer GENPAK 064008	24.3	Breast Cancer Res. Gen. 1024	15.6
97773 Ovarian cancer (OD06145)	0.0	85975 Breast Cancer (OD04590-01)	0.0
97775 Ovarian cancer NAT (OD06145)	0.0	85976 Breast Cancer Mets (OD04590-03)	0.0
98853 Ovarian cancer (OD06455-03)	0.0	87070 Breast Cancer Metastasis (OD04655-05)	0.0

98854 Ovarian NAT (OD06455-07) Fallopian tube	0.0	GENPAK Breast Cancer 064006	0.0
Normal Lung GENPAK 061010	0.0	Breast Cancer Clontech 9100266	8.0
92337 Invasive poor diff. lung adeno (ODO4945-01)	13.4	Breast NAT Clontech 9100265	0.0
92338 Lung NAT (ODO4945- 03)	0.0	Breast Cancer INVITROGEN A209073	0.0
84136 Lung Malignant Cancer (OD03126)	7.4	Breast NAT INVITROGEN A2090734	0.0
84137 Lung NAT (OD03126)	0.0	97763 Breast cancer (OD06083)	0.0
90372 Lung Cancer (OD05014A)	0.0	97764 Breast cancer node metastasis (OD06083)	0.0
90373 Lung NAT (OD05014B)	5.2	Normal Liver GENPAK 061009	0.0
97761 Lung cancer (OD06081)	0.0	Liver Cancer Research Genetics RNA 1026	0.0
97762 Lung cancer NAT (OD06081)	0.0	Liver Cancer Research Genetics RNA 1025	7.3
85950 Lung Cancer (OD04237- 01)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6004- T	0.0
85970 Lung NAT (OD04237- 02)	0.0	Paired Liver Tissue Research Genetics RNA 6004-N	0.0
83255 Ocular Mel Met to Liver (ODO4310)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6005- T	0.0
83256 Liver NAT (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
84139 Melanoma Mets to Lung (OD04321)	0.0	Liver Cancer GENPAK 064003	0.0
84138 Lung NAT (OD04321)	0.0	Normal Bladder GENPAK 061001	0.0
Normal Kidney GENPAK 061008	0.0	Bladder Cancer Research Genetics RNA 1023	0.0
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	Bladder Cancer INVITROGEN A302173	0.0
83787 Kidney NAT (OD04338)	0.0	Normal Stomach GENPAK 061017	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Gastric Cancer Clontech 9060397	0.0
83789 Kidney NAT (OD04339)	0.0	NAT Stomach Clontech 9060396	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	Gastric Cancer Clontech 9060395	0.0
83791 Kidney NAT (OD04340)	0.0	NAT Stomach Clontech 9060394	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Gastric Cancer GENPAK 064005	0.0

Panel 1.3D Summary Ag2494/Ag2345 Two experiments with two different probe and primer sets show highest expression of the CG50217-01 gene in a sample derived from lung cancer cell line and a gastric cancer cell line. This result suggests that expression of the CG50217-01 gene could be used to differentiate between lung and gastric cancer cell lines and other tissues and to detect the presence of gastric and lung cancers. Moreover, therapeutic modulation of the expression or function of this gene, through the use of small molecule drugs or antibodies, might be of use in the treatment of gastric and lung cancers.

Panel 2.2 Summary Ag2345 Significant expression of the CG50217-01 gene is limited to normal prostate tissue adjacent to a prostate cancer (CT=33.5). The gene appears to be overexpressed in normal prostate tissue when compared to the adjacent tumor. Thus, therapeutic upregulation of the activity of the CG50217-01 gene product, through the application of the protein product or agonists might be of use in the treatment of prostate cancer.

Panel 4D Summary Ag2494/Ag2345 Expression of the CG50217-0 in this panel is low/undetectable (CT values >35) in all samples (data not shown).

Example 3. SNP analysis of GPCRX clones

SeqCalling™ Technology: cDNA was derived from various human samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained as whole tissue, cell lines, primary cells or tissue cultured primary cells and cell lines. Cells and cell lines may have been treated with biological or chemical agents that regulate gene expression for example, growth factors, chemokines, steroids. The cDNA thus derived was then sequenced using CuraGen's proprietary SeqCalling technology. Sequence traces were evaluated manually and edited for corrections if appropriate. cDNA sequences from all samples were assembled with themselves and with public ESTs using bioinformatics programs to generate CuraGen's human SeqCalling database of SeqCalling assemblies. Each assembly contains one or more overlapping cDNA sequences derived from one or more human samples. Fragments and ESTs were included as components for an assembly when the extent of identity with another component of the assembly was at least 95% over 50 bp. Each assembly can represent a gene and/or its variants such as splice forms and/or single nucleotide polymorphisms (SNPs) and their combinations.

Variant sequences are included in this application. A variant sequence can include a single nucleotide polymorphism (SNP). A SNP can, in some instances, be referred to as a "cSNP" to denote that the nucleotide sequence containing the SNP originates as a cDNA. A SNP can arise in several ways. For example, a SNP may be due to a substitution of one nucleotide for another at the polymorphic site. Such a substitution can be either a transition or a transversion. A SNP can also arise from a deletion of a nucleotide or an insertion of a nucleotide, relative to a reference allele. In this case, the polymorphic site is a site at which one allele bears a gap with respect to a particular nucleotide in another allele. SNPs occurring within genes may result in an alteration of the amino acid encoded by the gene at the position of the SNP. Intragenic SNPs may also be silent, however, in the case that a codon including a SNP encodes the same amino acid as a result of the redundancy of the genetic code. SNPs occurring outside the region of a gene, or in an intron within a gene, do not result in changes in any amino acid sequence of a protein but may result in altered regulation of the expression pattern for example, alteration in temporal expression, physiological response regulation, cell type expression regulation, intensity of expression, stability of transcribed message.

Method of novel SNP Identification: SNPs are identified by analyzing sequence assemblies using CuraGen's proprietary SNPTool algorithm. SNPTool identifies variation in assemblies with the following criteria: SNPs are not analyzed within 10 base pairs on both ends of an alignment; Window size (number of bases in a view) is 10; The allowed number of mismatches in a window is 2; Minimum SNP base quality (PHRED score) is 23; Minimum number of changes to score an SNP is 2/assembly position. SNPTool analyzes the assembly and displays SNP positions, associated individual variant sequences in the assembly, the depth of the assembly at that given position, the putative assembly allele frequency, and the SNP sequence variation. Sequence traces are then selected and brought into view for manual validation. The consensus assembly sequence is imported into CuraTools along with variant sequence changes to identify potential amino acid changes resulting from the SNP sequence variation. Comprehensive SNP data analysis is then exported into the SNPCalling database.

Method of novel SNP Confirmation: SNPs are confirmed employing a validated method known as Pyrosequencing (Pyrosequencing, Westborough, MA). Detailed protocols for Pyrosequencing can be found in: Alderborn et al. Determination of Single Nucleotide Polymorphisms by Real-time Pyrophosphate DNA Sequencing. (2000). *Genome Research*. 10, Issue 8, August. 1249-1265. In brief, Pyrosequencing is a real time primer extension process

of genotyping. This protocol takes double-stranded, biotinylated PCR products from genomic DNA samples and binds them to streptavidin beads. These beads are then denatured producing single stranded bound DNA. SNPs are characterized utilizing a technique based on an indirect bioluminometric assay of pyrophosphate (PPi) that is released from each dNTP upon DNA chain elongation. Following Klenow polymerase-mediated base incorporation, PPi is released and used as a substrate, together with adenosine 5'-phosphosulfate (APS), for ATP sulfurylase, which results in the formation of ATP. Subsequently, the ATP accomplishes the conversion of luciferin to its oxi-derivative by the action of luciferase. The ensuing light output becomes proportional to the number of added bases, up to about four bases. To allow processivity of the method dNTP excess is degraded by apyrase, which is also present in the starting reaction mixture, so that only dNTPs are added to the template during the sequencing. The process has been fully automated and adapted to a 96-well format, which allows rapid screening of large SNP panels. The DNA and protein sequences for the novel single nucleotide polymorphic variants are reported. Variants are reported individually but any combination of all or a select subset of variants are also included. In addition, the positions of the variant bases and the variant amino acid residues are underlined.

GPCR1 Results

GPCR1a

The DNA and protein sequences for the novel single nucleotide polymorphic variants of the GPCR-like gene of CuraGen Acc. No. GMAC073079_A_ are reported. In summary, there are 13 variants. Variant 13373946 is a C to T SNP at 100 bp of the nucleotide sequence that results in a Pro to Ser change at amino acid 28 of protein sequence, variant 13374313 is an A to G SNP at 221 bp of the nucleotide sequence that results in a Glu to Gly change at amino acid 68 of protein sequence, variant 13373944 is a T to C SNP at 268 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374312 is a T to C SNP at 272 bp of the nucleotide sequence that results in a Val to Ala change at amino acid 85 of protein sequence, variant 13374311 is a C to T SNP at 318 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13373942 is a T to C SNP at 346 bp of the nucleotide sequence that results in a Phe to Leu change at amino acid 110 of protein sequence, variant 13374001 is a G to A SNP at 444 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374309 is a C to T SNP at 472

bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374307 is a C to T SNP at 534 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374306 is a T to C SNP at 563 bp of the nucleotide sequence that results in a Leu to Pro change at amino acid 182 of protein sequence, variant 13374305 is a C to A SNP at 663 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13373937 is a C to T SNP at 728 bp of the nucleotide sequence that results in a Ser to Phe change at amino acid 237 of protein sequence, and variant 13373936 is a C to T SNP at 928 bp of the nucleotide sequence that results in a Gln to Stop at amino acid 304 of protein sequence.

GPCR1b

There are 14 variants reported for GPCR1b. Variant 13374122 is a C to T SNP at 105 bp of the nucleotide sequence that results in a Pro to Ser change at amino acid 26 of protein sequence, variant 13374313 is an A to G SNP at 226 bp of the nucleotide sequence that results in a Glu to Gly change at amino acid 66 of protein sequence, variant 13373944 is a T to C SNP at 273 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374312 is a T to C SNP at 277 bp of the nucleotide sequence that results in a Val to Ala change at amino acid 83 of protein sequence, variant 13374311 is a C to T SNP at 323 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13373942 is a T to C SNP at 351 bp of the nucleotide sequence that results in a Phe to Leu change at amino acid 108 of protein sequence, variant 13374001 is a G to A SNP at 449 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374309 is a C to T SNP at 477 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13373940 is an insertion of nucleotide T after 514 bp of the nucleotide sequence that results in a frameshift with all amino acids after 162 being discordant with the original protein sequence, variant 13374307 is a C to T SNP at 539 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374306 is a T to C SNP at 568 bp of the nucleotide sequence that results in a Leu to Pro change at amino acid 180 of protein sequence, variant 13373938 is an A to C SNP at 668 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13373937 is a C to T SNP at 733 bp of the nucleotide sequence that results in a Ser to Phe change at amino acid 235 of protein sequence, and variant 13373936 is a C to T SNP at 933 bp

of the nucleotide sequence that results in a Gln to Stop at amino acid 302 of protein sequence.
Additional GPCR1 SNPs are provided in Table 27A below.

Table 27A: Additional GPCR1 SNPs

GPCR1b SNPs:				
Cons.Pos.: 166	Depth: 23	Change: C > T	Putative Allele Freq.:	0.087
Cons.Pos.: 172	Depth: 23	Change: T > C	Putative Allele Freq.:	0.217
GPCR1c SNPs:				
Cons.Pos.: 33	Depth: 18	Change: C > A	Putative Allele Freq.:	0.111
Cons.Pos.: 279	Depth: 31	Change: T > C	Putative Allele Freq.:	0.065
Cons.Pos.: 447	Depth: 41	Change: C > -	Putative Allele Freq.:	0.
Cons.Pos.: 453	Depth: 40	Change: G > A	Putative Allele Freq.:	0.050
Cons.Pos.: 673	Depth: 25	Change: C > A	Putative Allele Freq.:	0.080
GPCR1d SNPs:				
Cons.Pos.: 40	Depth: 24	Change: G > A	Putative Allele Freq.:	0.083
Cons.Pos.: 63	Depth: 51	Change: T > -	Putative Allele Freq.:	0.039
Cons.Pos.: 66	Depth: 51	Change: T > -	Putative Allele Freq.:	0.039
Cons.Pos.: 250	Depth: 51	Change: G > A	Putative Allele Freq.:	0.098
Cons.Pos.: 315	Depth: 51	Change: G > T	Putative Allele Freq.:	0.039
Cons.Pos.: 415	Depth: 58	Change: A > G	Putative Allele Freq.:	0.034
Cons.Pos.: 434	Depth: 65	Change: A > -	Putative Allele Freq.:	0.031
Cons.Pos.: 443	Depth: 66	Change: T > -	Putative Allele Freq.:	0.030
Cons.Pos.: 444	Depth: 66	Change: G > -	Putative Allele Freq.:	0.030
Cons.Pos.: 444	Depth: 66	Change: G > A	Putative Allele Freq.:	0.091
Cons.Pos.: 470	Depth: 75	Change: A > G	Putative Allele Freq.:	0.027
Cons.Pos.: 495	Depth: 78	Change: G > -	Putative Allele Freq.:	0.026
Cons.Pos.: 507	Depth: 79	Change: G > A	Putative Allele Freq.:	0.025
Cons.Pos.: 535	Depth: 77	Change: C > T	Putative Allele Freq.:	0.026
Cons.Pos.: 663	Depth: 50	Change: G > A	Putative Allele Freq.:	0.060
Cons.Pos.: 709	Depth: 51	Change: A > G	Putative Allele Freq.:	0.039
Cons.Pos.: 760	Depth: 51	Change: T > C	Putative Allele Freq.:	0.039
Cons.Pos.: 886	Depth: 51	Change: G > A	Putative Allele Freq.:	0.059
Cons.Pos.: 959	Depth: 25	Change: G > T	Putative Allele Freq.:	0.080
GPCR1e SNPs:				
Cons.Pos.: 33	Depth: 39	Change: C > A	Putative Allele Freq.:	0.051
Cons.Pos.: 105	Depth: 65	Change: C > T	Putative Allele Freq.:	0.046
Cons.Pos.: 226	Depth: 65	Change: A > G	Putative Allele Freq.:	0.031
Cons.Pos.: 277	Depth: 65	Change: T > C	Putative Allele Freq.:	0.031
Cons.Pos.: 323	Depth: 64	Change: C > T	Putative Allele Freq.:	0.047
Cons.Pos.: 449	Depth: 88	Change: G > A	Putative Allele Freq.:	0.023
Cons.Pos.: 477	Depth: 86	Change: C > T	Putative Allele Freq.:	0.023
Cons.Pos.: 514	Depth: 82	Change: T > C	Putative Allele Freq.:	0.02
Cons.Pos.: 539	Depth: 75	Change: C > T	Putative Allele Freq.:	0.107
Cons.Pos.: 568	Depth: 67	Change: T > C	Putative Allele Freq.:	0.030
Cons.Pos.: 668	Depth: 60	Change: C > A	Putative Allele Freq.:	0.033
Cons.Pos.: 733	Depth: 61	Change: C > T	Putative Allele Freq.:	0.115
GPCR1f SNPs:				

Cons.Pos.: 54 Depth: 39 Change: C > A AA translation view (alpha) Fragment Listing: -> 145919905(+,i,119650936) Fpos: 134 -> 145919950(+,i,119650936) Fpos: 110	Putative Allele Freq.: 0.051
Cons.Pos.: 127 Depth: 65 Change: C > T AA translation view (alpha) Fragment Listing: -> 146985611(+,i,119650936) Fpos: 122 -> 146985939(+,i,119650936) Fpos: 118 -> 146990206(+,i,119650936) Fpos: 116	Putative Allele Freq.: 0.046
Cons.Pos.: 253 Depth: 65 Change: A > G AA translation view (alpha) Fragment Listing: -> 147090582(+,i,119650936) Fpos: 282 -> 147090676(+,i,119650936) Fpos: 274	Putative Allele Freq.: 0.031
Cons.Pos.: 304 Depth: 65 Change: T > C AA translation view (alpha) Fragment Listing: -> 146170427(+,i,119650936) Fpos: 305 -> 146170442(+,i,119650936) Fpos: 299	Putative Allele Freq.: 0.031
Cons.Pos.: 350 Depth: 64 Change: C > T AA translation view (alpha) Fragment Listing: -> 146985893(+,i,119650936) Fpos: 340 -> 146985897(+,i,119650936) Fpos: 344 -> 147090633(+,i,119650936) Fpos: 384	Putative Allele Freq.: 0.047
Cons.Pos.: 472 Depth: 90 Change: C > - AA translation view (alpha) Fragment Listing: -> 146170397(+,i,119650936) Fpos: 467 -> 146170474(+,i,119650936) Fpos: 467	Putative Allele Freq.: 0.022
Cons.Pos.: 478 Depth: 88 Change: G > A AA translation view (alpha) Fragment Listing: -> 145919950(+,i,119650936) Fpos: 526 -> 146115328(-,i,119650936) Fpos: 580	Putative Allele Freq.: 0.023
Cons.Pos.: 506 Depth: 86 Change: C > - AA translation view (alpha) Fragment Listing: -> 146170474(+,i,119650936) Fpos: 500 -> 147090702(-,i,119650936) Fpos: 541	Putative Allele Freq.: 0.023
Cons.Pos.: 506 Depth: 86 Change: C > T AA translation view (alpha) Fragment Listing: -> 146952987(+,i,119650936) Fpos: 519 -> 146985961(-,i,119650936) Fpos: 512	Putative Allele Freq.: 0.023
Cons.Pos.: 518 Depth: 84 Change: C > - AA translation view (alpha)	Putative Allele Freq.: 0.024


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Fragment Listing:
-> 146170540(+,i,119650936) Fpos: 519
-> 147191380(+,i,119650936) Fpos: 587

Cons.Pos.: 544 Depth: 82 Change: T > C Putative Allele Freq.: 0.024
AA translation view (alpha)
Fragment Listing:
-> 146952753(-,i,119650936) Fpos: 487
-> 146985831(+,i,119650936) Fpos: 528

Cons.Pos.: 570 Depth: 75 Change: C > T Putative Allele Freq.: 0.107
AA translation view (alpha)
Fragment Listing:
-> 146115375(-,i,119650936) Fpos: 489
-> 146952753(-,i,119650936) Fpos: 462
-> 146952861(-,i,119650936) Fpos: 484
-> 146952886(-,i,119650936) Fpos: 484
-> 146985831(+,i,119650936) Fpos: 553
-> 146985913(-,i,119650936) Fpos: 464
-> 147191402(-,i,119650936) Fpos: 506
-> 147191417(-,i,119650936) Fpos: 512

Cons.Pos.: 572 Depth: 75 Change: A > - Putative Allele Freq.: 0.053
AA translation view (alpha)
Fragment Listing:
-> 146115265(+,i,119650936) Fpos: 594
-> 146952642(+,i,119650936) Fpos: 574
-> 146985893(+,i,119650936) Fpos: 557
-> 146985897(+,i,119650936) Fpos: 561

Cons.Pos.: 581 Depth: 74 Change: T > - Putative Allele Freq.: 0.027
AA translation view (alpha)
Fragment Listing:
-> 146952642(+,i,119650936) Fpos: 582
-> 146985897(+,i,119650936) Fpos: 569

Cons.Pos.: 599 Depth: 67 Change: T > C Putative Allele Freq.: 0.030
AA translation view (alpha)
Fragment Listing:
-> 146985611(+,i,119650936) Fpos: 585
-> 146990206(+,i,119650936) Fpos: 578

Cons.Pos.: 699 Depth: 60 Change: C > A Putative Allele Freq.: 0.033
AA translation view (alpha)
Fragment Listing:
-> 145919890(-,i,119650936) Fpos: 389
-> 146115328(-,i,119650936) Fpos: 361

Cons.Pos.: 764 Depth: 61 Change: C > T Putative Allele Freq.: 0.115
AA translation view (alpha)
Fragment Listing:
-> 146952735(-,i,119650936) Fpos: 287
-> 146952816(-,i,119650936) Fpos: 286
-> 146952861(-,i,119650936) Fpos: 290
-> 146952886(-,i,119650936) Fpos: 290
-> 146985913(-,i,119650936) Fpos: 270
-> 147191417(-,i,119650936) Fpos: 318
-> 147191539(-,i,119650936) Fpos: 311

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Cons.Pos.: 948 Depth: 61 Change: A > - Putative Allele Freq.: 0.049
AA translation view (alpha)
Fragment Listing:
-> 146952735(-,i,119650936) Fpos: 112
-> 147090580(-,i,119650936) Fpos: 112
-> 147090702(-,i,119650936) Fpos: 114

Cons.Pos.: 974 Depth: 34 Change: C > T Putative Allele Freq.: 0.118
AA translation view (alpha)
Fragment Listing:
-> 147090647(-,i,119650936) Fpos: 89
-> 147090662(-,i,119650936) Fpos: 101
-> 147191500(-,i,119650936) Fpos: 104
-> 147191579(-,i,119650936) Fpos: 115

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GPCR2

5 Variants are reported individually but any combination of all or a select subset of
variants are also included.

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Cons.Pos.: 460 Depth: 27 Change: T > - Putative Allele Freq.: 0.111
Cons.Pos.: 496 Depth: 29 Change: T > - Putative Allele Freq.: 0.069
Cons.Pos.: 623 Depth: 19 Change: T > - Putative Allele Freq.: 0.158
Cons.Pos.: 638 Depth: 18 Change: T > - Putative Allele Freq.: 0.111
10 Cons.Pos.: 834 Depth: 14 Change: C > T Putative Allele Freq.: 0.143
Cons.Pos.: 854 Depth: 14 Change: C > - Putative Allele Freq.: 0.429
Cons.Pos.: 859 Depth: 14 Change: C > - Putative Allele Freq.: 0.429

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GPCR3

15 AS summarized below in Tables 27B and 27C, there are multiple variants reported for
GPCR3. As shown in Table 27C, variant 13375479 is an A to G SNP at 71 bp of the
nucleotide sequence that results in no change in the protein sequence (silent), variant
13375480 is a C to T SNP at 185 bp of the nucleotide sequence that results in no change in the
protein sequence (silent), variant 13375481 is a G to C SNP at 389 bp of the nucleotide
20 sequence that results in an Arg to Ser change at amino acid 129 of protein sequence, variant
13375482 is a G to A SNP at 541 bp of the nucleotide sequence that results in a Cys to Tyr
change at amino acid 180 of protein sequence, variant 13375483 is a T to C SNP at 780 bp of
the nucleotide sequence that results in a Tyr to His change at amino acid 260 of protein
sequence, variant 13375484 is a T to C SNP at 858 bp of the nucleotide sequence that results
25 in no change in the protein sequence (silent), and variant 13375485 is a G to C SNP at 867 bp
of the nucleotide sequence that results in a Val to Leu change at amino acid 289 of protein

sequence.

Table 27B. cSNP for GPCR3				
Cons. Pos.	Depth	Wild Type	Variant	Putative Allele Frequency
495	35	C	-	0.057
502	34	C	-	0.059
862	13	T	C	0.308
871	13	G	C	0.308

Table 27C. cSNP for GPCR3				
Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13375479	71	A	G	None
13375480	185	C	T	None
13375481	389	G	C	Arg -> Ser
13375482	541	G	A	Cys -> Tyr
13375483	780	T	C	Tyr -> His
13375484	858	T	C	None
13375485	867	G	C	Val -> Leu

5 GPCR4

As summarized below in Tables 27E and 27F, there are multiple variants reported for GPCR4a. As shown in Table 27F, variant 13373774 is a C to T SNP at 206 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13019736 is a T to C SNP at 210 bp of the nucleotide sequence that results in a Tyr to His change at amino acid 60 of protein sequence, variant 13373983 is a C to G SNP at 389 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374293 is a T to C SNP at 390 bp of the nucleotide sequence that results in a Tyr to His change at amino acid 120 of protein sequence, variant 13374292 is an A to G SNP at 430 bp of the nucleotide sequence that results in a His to Arg change at amino acid 133 of protein sequence, variant 13374291 is a T to C SNP at 523 bp of the nucleotide sequence that results in a Met to Thr change at amino acid 164 of protein sequence, variant 13375488 is a C to T SNP at 536 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374290 is a C to T SNP at 542 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13373986 is a G to A SNP at 551 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant

13373987 is a G to A SNP at 579 bp of the nucleotide sequence that results in an Ala to Thr change at amino acid 183 of protein sequence, variant 13375487 is a C to T SNP at 630 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant 13374288 is an A to G SNP at 702 bp of the nucleotide sequence that results in a Thr to Ala change at amino acid 224 of protein sequence, variant 13373989 is a C to T SNP at 760 bp of the nucleotide sequence that results in an Ala to Val change at amino acid 243 of protein sequence, and variant 13373990 is a G to C SNP at 960 bp of the nucleotide sequence that results in an Ala to Pro change at amino acid 310 of protein sequence.

Table 27E. cSNPs GPCR4a

Cons. Pos.	Depth	Wild Type	Variant
209	105	C	T
213	105	T	C
393	109	C	G
394	109	T	C
435	134	G	A
490	152	C	-
496	156	C	-
503	158	A	-
504	158	A	-
513	162	C	-
533	160	T	C
552	155	C	T
561	148	G	A
582	132	A	-
591	130	G	A
638	99	C	-
645	94	G	-
652	93	C	-
657	93	C	-
658	93	C	-
715	94	A	G
773	95	C	T
978	88	G	C

Table 27F. cSNP for GPCR4a

Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13373774	206	C	T	None
13019736	210	T	C	Tyr -> His
13373983	389	C	G	None
13374293	390	T	C	Tyr -> His
13374292	430	A	G	His -> Arg
13374291	523	T	C	Met -> Thr
13375488	536	C	T	None
13374290	542	C	T	None
13373986	551	G	A	None

13373987	579	G	A	Ala -> Thr
13375487	630	C	T	None
13374288	702	A	G	Thr -> Ala
13373989	760	C	T	Ala -> Val
13373990	960	G	C	Ala -> Pro

As summarized below in Table 27G, there are multiple variants reported for GPCR4b.

Table 27G. cSNP for GPCR4b				
Cons. Pos.	Depth	Wild Type	Variant	Putative Allele Frequency
451	33	C	T	0.061
490	33	G	A	0.061
602	20	C	T	0.350
826	21	G	A	0.286

5 GPCR5

A summarized below in Table 27H, there are several variants reported for GPCR5. As shown in Table 27H, variant 13375490 is an A to G SNP at 1380 bp of the nucleotide sequence that results in no change in the protein sequence (silent), and variant 13375489 is a C to T SNP at 1832 bp of the nucleotide sequence that results in no change in the protein sequence since the SNP is not in the amino acid coding region.

Table 27H. cSNP for GPCR5				
Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13375490	1380	A	G	None
13375489	1832	C	T	None

GPCR6

As shown below in Table 27I, variant 13373922 is a G to A SNP at 299 bp of the nucleotide sequence that results in an Ala to Thr change at amino acid 100 of protein sequence, variant 13373923 is an A to G SNP at 401 bp of the nucleotide sequence that results in a Thr to Ala change at amino acid 134 of protein sequence, and variant 13373924 is a T to A SNP at 459 bp of the nucleotide sequence that results in a Leu to His change at amino acid 153 of protein sequence.

Table 27I. cSNPs for GPCR6

Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13373922	299	G	A	Ala -> Thr
13373923	401	A	G	Thr -> Ala
13373924	459	T	A	Leu -> His

GPCR7

As shown below in Table 27J, variant 13373933 is a C to T SNP at 128 bp of the nucleotide sequence that results in no change in the protein sequence (silent), variant

- 5 13373934 is a C to G SNP at 383 bp of the nucleotide sequence that results in an Ile to Met change at amino acid 127 of protein sequence, and variant 13373935 is a T to C SNP at 813 bp of the nucleotide sequence that results in a Trp to Arg change at amino acid 271 of protein sequence.

Table 27J. cSNPs for GPCR7				
Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13373933	128	C	T	No change
13373934	383	C	G	Ile -> Met
13373935	813	T	C	Trp -> Arg

GPCR8

As summarized below in Table 27K, there is one variant reported for GPCR8. As shown in Table 27K, variant 13375497 is a T to C SNP at 263 bp of the nucleotide sequence that results in an Ile to Thr change at amino acid 77 of the protein sequence.

Table 27K. cSNP for GPCR8				
Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13375497	263	T	C	Ile -> Thr

GPCR9

As summarized below in Table 27L, there is one variant reported for GPCR9. As shown in Table 27L, variant 13375498 is a G to A SNP at 839 bp of the nucleotide sequence that results in no change in the protein sequence (silent).

Table 27L. cSNP for GPCR9				
Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13375498	839	G	A	None

GPCR10

Possible SNPs found for GPCR10a are listed in Table 27M. Putative Allele Frequency (PAF) is shown in column four.

Table 27M: SNPs			
Consensus Position	Depth	Base Change	PAF
634	14	T > G	0.357

GPCR11

Cons.Pos.: 505 Depth: 39 Change: G > A

Putative Allele Freq.: 0.051

Cons.Pos.: 811 Depth: 33 Change: G > A

Putative Allele Freq.: 0.091

GPCR12

As summarized below in Tables 27N and 27O, there are multiple variants reported for GPCR12. As shown in Table 27N, variant 13373913 is a C to T SNP at 223 bp of the nucleotide sequence that results in a Leu to Phe change at amino acid 75 of protein sequence; variant 13373912 is a T to G SNP at 730 bp of the nucleotide sequence that results in a Ser to Ala change at amino acid 244 of protein sequence; and variant 13373911 is a G to A SNP at 805 bp of the nucleotide sequence that results in an Asp to Asn change at amino acid 269 of protein sequence.

Table 27N. cSNP for GPCR12

Cons. Pos.	Depth	Wild Type	Variant	Putative Allele Frequency
505	39	G	A	0.051
811	33	G	A	0.091

Table 27O. cSNP for GPCR12				
Name of Variant	Base Position of cSNP	Wild Type	Variant	Amino Acid Change
13373913	233	C	T	Leu -> Phe
13373912	730	T	G	Ser -> Ala
13373911	805	G	A	Asp -> Asn

EQUIVALENTS

- 5 Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, which follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined
- 10 by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims.